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# THE PROBLEM OF INFECTION AS PRESENTED BY BACTERIAL INVASION OF THE CHORIO-ALLANTOIC MEMBRANE OF CHICK EMBRYOS *

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Three years ago it was suggested by one of us that the chorio-allantoic membrane of chick embryos might be serviceable as a sterile living culture medium for the study of bacterial infection.[1] Since that time we have had occasion to inoculate the membrane with a variety of bacteria in pure culture and to observe some of the effects that infection presents. Results of these initial studies seem to us worthy of record as a survey of the method, because they indicate the practicability of utilizing this living host for the propagation of pure cultures and for studying the interaction between the host and parasite under rather uniform conditions.

The method of isolating and studying pure cultures of bacteria on dead media has yielded a great fund of information regarding the cultivable microorganisms, but one is often quite sure that prolonged or even brief growth of these pathogenic agents under such artificial conditions may alter in some way their disease-inducing potentialities. For the preparation of vaccines the utilization of freshly isolated cultures has been emphasized; and a routine means for maintaining the immunizing qualities of a particular strain of bacteria is certainly desirable. If the infectiousness of a particular bacterium is

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to be tested it must be introduced into a living host, and it is usually the terminal effects that are likely to offer themselves most readily for observation, so that the host-parasite relation during invasion remains obscure. The initial stages of infection are of great importance in an understanding of the state of parasitism, yet by the usual methods the period of invasion and the early reaction of the host are not easily submitted to observation.

Experimental animals that have maintained an independent existence for an indefinite period under variable and complex conditions are not by any means to be considered, we believe, so uniform a living medium for the study of infection as an embryo and its tissues, which have not yet been subjected to the varying influences of bacterial infestation, protective responses, dietary changes, maturation and other incidents of independent life.

With these and other considerations in mind we have undertaken to infect the embryonic chick membrane with several species of bacteria in order to observe the course of events at the site of inoculation at various intervals, and to form a judgment as to some of the possibilities this technique might offer in the study of problems of bacterial infection and host resistance. For this purpose we have used pure cultures of *Staph. aureus*, *Str. haemolyticus*, *Str. viridans*, *A. aerogenes*, *E. typhi*, *Br. abortus*, *C. diphtheriae*, and *Myco. tuberculosis avium*.

We have observed that all of these pathogenic bacteria, with the exception of *Staph. aureus*, *Str. haemolyticus* and *C. diphtheriae*, are able to multiply within the protoplasm of the embryonic cells, utilizing those of mesodermal derivation or those of the epithelial layers, or both, for growth in the process of invasion. All of these microorganisms will grow extracellularly in the presence of necrosis but they do not seem to invade the living tissues except by means of viable cells as a vehicle. Phagocytosis under these circumstances instead of defending the host, offers an acceptable mechanism for invasion. Susceptibility of this host means at least in part the availability of living cells as a medium for bacterial growth. Phagocytosis, aside from the physical problem involved, has thus an ambiguous significance. It may result in the destruction of the invader or it may provide it a hospitable reception. These and other problems are considered in the discussion.

## Technique

The general technique employed has been described in a previous publication from this laboratory in a study of the cultivation of vaccinia virus.[2] In order to observe various stages in the progress of infection the "cover-slip" or "window" method has been used.

Smears were routinely stained by Wright's method and on occasion by that of Gram. Tissues were usually fixed in a modified Schaudinn's fluid, consisting of 2 parts of saturated aqueous solution of corrosive sublimate and 1 part of 60 per cent alcohol.

Microscopic sections were stained routinely by the hematoxylin-eosin method and by means of Wright's stain. The method for the latter was as follows: Soloid tablets were dissolved in methyl alcohol (1 tablet to 10 cc.). Sixty drops, from a dropping bottle, of the stock solution were added to 100 cc. of distilled water. Sections were stained 2 to 3 hours by emersion in the fluid. They were differentiated rapidly in absolute ethyl alcohol, cleared in xylol and mounted in cedar oil. Sections 4 $\mu$ thick, properly stained, give an excellent differentiation of both bacteria and cells.

### *Staphylococcus aureus*

*Strain I:* Isolated from a human brain abscess by the blood agar plate method. For inoculating the membranes a 24 hour broth culture was employed and 1 drop of the broth from a capillary pipette was placed on each membrane of 6 14 day embryos.

After 48 hours there was a slight gray exudative lesion at the site of the inoculation. Smears were made at intervals. Specimens were killed and fixed for histological study at 48, 72 and 96 hours. The remaining embryos lived to hatch about the 21st day of incubation.

Only the membranal lesions were studied in the case of embryos inoculated with *Staph. aureus*. The embryos were not autopsied.

*Strain II:* Isolated from a lung abscess in a child. A 24 hour broth culture was used to inoculate 4 14 day embryos with 1 drop each from a capillary pipette. After 24 hours there was massive growth of the organisms. At 48 hours 2 embryos were dead. The remaining 2 lived only a few hours longer. Transfers were made to other embryos by inoculating the membrane with a platinum loop of bacteria and exudate. Infection took place in the second genera-

tion killing the embryos in 48 hours. Abundant purulent exudate developed on the membranes.

*Strain III:* Isolated from cutaneous lesions of a case of impetigo contagiosum. This strain was inoculated on the membrane of a 10 day embryo in the form of a saline suspension of aspirated material from the human lesion in saline. For the inoculum 0.2 cc. was used.

After 24 hours there were four small white nodules in the inoculated area. After 48 hours there was graying, thickening and opacity of the membrane, which advanced along the blood vessels. At this time the lesion was removed and fixed for histological study.

Small fragments of this membrane were used to inoculate 5 9 day embryos and culture media. Cultures yielded pure *Staph. aureus*, and after 4 days 3 embryos had died and 2 survived.

Smears from the membranal lesions were made at intervals and stained by Gram's and Wright's methods. Smear preparations made from membranal exudate 24 hours after inoculation presented the following picture.

*Eosinophiles:* The cellular exudate is abundant. The predominant cell contains irregular eosinophilic granules. No definite rod shaped granules are seen. These eosinophilic cells are of two chief varieties, a smaller polymorphonuclear cell and a larger mononuclear cell. They are interpreted to be polymorphonuclear leukocytes and myelocytes respectively. The eosinophilic granular material appears to be unstable. The granules vary greatly in size and seem to fuse. In some cells that contain bacteria the eosinophilic material has completely disappeared; in others it seems to have dissolved or liquefied and flowed about, or adhered to the bacteria, so that the staining effect suggests a thin coating of the cocci by this material. In relatively few instances does one find discrete eosinophilic granules and cocci in the same cell.

*Mononuclears:* The second most numerous cell is a mononuclear, non-granular leukocyte with round, oval or indented nucleus and abundant basophilic cytoplasm. The cytoplasm commonly has an irregular or scalloped margin with the protrusion of pseudopods of cytoplasm. The cytoplasm of these cells is characteristically vacuolated. The vacuoles in the smaller cells are round and sharply defined, varying in size. In the larger cells the vacuoles are larger and more indefinite in outline. Cells similar to these sometimes contain a few basophilic granules.

*Bacteria and Phagocytosis:* Numerous cocci are present mostly within cells. The polymorphonuclears are responsible at this stage for the greatest phagocytic activity. Within the leukocytes the cocci are commonly very faintly staining and smaller than normal, as if undergoing degeneration. With them one often sees a few deeply staining (blue) forms which seem unaffected. Small groups of cocci are extracellular. Occasional large mononuclears have engulfed other cells.

*Microscopic Sections 48 Hours After Inoculation:* Purulent exudate, in which discrete colonies of cocci are embedded, is abundant. The exudate is fusing and becoming hyalinized next to the epithelial layer. A few pustules are situated within the epithelial layer.

Where the surface is ulcerated the exudate lies on a granulating membrane and there is a layer of foreign body giant cells which almost completely separates the two. Polymorphonuclears are for the most part eosinophilic, but no distinct rod shaped granules are seen. Myelocytes are proliferating in the thickened membrane. Fibroblasts beneath the epithelium and exudate are hyperplastic.

*Comment:*

*Staph. aureus* grows abundantly on the surface of the membrane, usually forming a discrete focus at the site of inoculation which tends in 2 or 3 days to become definitely circumscribed and reduced in diameter by contraction of the membrane. The cocci usually remain localized in the form of discrete rounded colonies embedded in the exudate, except where the membrane becomes necrotic. Over the necrotic area a film of growth is present.

Growth of the microorganism is characteristically extracellular, and superficial necrosis of epithelium results. The cocci are readily phagocytosed by polymorphonuclear leukocytes, and to a less extent by large mononuclear cells. Many phagocytosed cocci undergo degeneration within the cytoplasm of phagocytes and there is no evidence of a tendency to reproduce within the cytoplasmic medium. Phagocytosis, however, is not the only and probably not the chief mechanism that resists invasion of the tissues. Colonies of cocci become surrounded by an exudate, the cells of which fuse and form what appears to be an impenetrable barrier about them. Under these conditions the cocci are held in check and undergo degenerative changes.

The leukocytic exudate incorporating the cocci is also walled off from the underlying tissue by a solid barrier of giant cells, beneath which regeneration of fibroblasts and epithelial cells progresses.

### *Streptococcus haemolyticus*

*Strain I:* A stock strain that has been maintained on artificial media 15 to 20 years. Isolated originally from a case of cellulitis at the Children's Hospital, Boston, Mass.

Inoculations were made by dropping 1 capillary drop of a 24 hour infusion broth culture onto the exposed embryonic membrane of 12 to 14 day old chick embryos.

At 24 hours after inoculation the membranes usually showed small, very thin, grayish white plaques of exudate over the inoculated surface. During this interval the organism had grown freely in chains from 4 to 14 units in length. The exudate containing the cocci could be easily picked up with a loop from the surface of the membrane. Smears made at 24 hours show free growth of the bacteria. There are red blood cells, and a leukocytic reaction in which polymorphonuclear eosinophiles predominate. These cells, however, do not readily phagocytose the cocci. A few mononuclear leukocytes may be present at this stage and may exhibit a slight degree of phagocytosis.

During the next 24 hours the gross lesion becomes thicker and more widely spread, often covering an area 8–10 mm. square. The lesion displays an opacity which tends to be cream colored. Its surface is moist. The organism has continued to grow. Smears show definite increase in the number of leukocytes. Polymorphonuclears are still present in large numbers but the increase in number and in the proportion of mononuclears is noticeable. In contrast to the absence of phagocytosis by the polymorphonuclears, the mononuclears may be actively phagocytozing the streptococci.

At from 48–72 hours after inoculation the lesion becomes localized. The margin of reaction is usually clearly outlined. The lesion becomes thicker and of a pus-like consistence and color. The appearance of the 72 hour lesion indicates whether or not the embryo is successfully combatting or succumbing to the invasion. Smears stained by Wright's method show an exudate made up almost entirely of large mononuclear leukocytes. There are a few inactive

polymorphonuclears and a few clasmatocytes active in engulfing débris. The mononuclears may have almost completely taken up the streptococci by phagocytosis or there may still be a good number of viable extracellular forms. In any case the mononuclears often become so filled with bacteria that the nuclei assume a thin crescent shape against the cell wall. Within the mononuclears the streptococci undergo notable degeneration. Different degrees may be demonstrated in one cell. Degeneration of bacterial cells is indicated by their pale blue to pale pink, shadowy and very small forms in contrast to the deep blue color of the normal cocci.

Shortly after 72 hours the lesion on the membrane which is overcoming the infection begins to dry. It becomes thick, wrinkled and scab-like. From 96 hours on, the process is one of apparent healing. Smears from a dry membrane are unsatisfactory but the organism is rarely if ever lost sight of completely. The organism will always grow out when bits of the lesion are inoculated onto a blood plate or into broth.

During experimentation with this strain 75 eggs were inoculated and observed. Transfers were made through five generations. Material from an infected membrane was plated out on blood agar. A broth tube was inoculated from the plate, and the 24 hour broth culture inoculated onto the embryonic membrane to give the next membranal generation.

A group of 10 embryos inoculated as described gives a representative mortality rate. One embryo was dead at 24 hours, an additional one at 48 hours, and 2 others at 96 hours. It is not unusual for embryos inoculated on the 13th or 14th day of incubation to hatch. Nor is it unusual for the embryo to die on the day it is due to hatch. Chicks that hatch are weak and gain strength slowly. Out of 11 hatched chicks autopsied at intervals of from 1 to 7 days, seven heart-blood cultures were positive for the presence of streptococcus. The organism was recovered from four spleens. Five of 6 embryos autopsied before hatching yielded the organism from the heart's blood.

An experiment involving rapid transfer of streptococcus directly from egg to egg was carried out. Transfers by loop from membrane to membrane were made at 24 hour intervals through thirteen successive passages. It was observed that younger embryos (10–12 days) offered a medium less resistant to growth of the organism than

older ones. There was no apparent change in cultural characteristics or pathogenicity of the strain after these rapid passages or after the slower passages.

*Strain II:* This strain was isolated April 4, 1935, from the heart's blood from a case of streptococcic arthritis of both knee joints with concurrent streptococcic septicemia. The cocci are very small, definitely hemolytic, and grow in long chains. One capillary drop of 24 hour infusion broth culture was used for the inoculum.

The lesion produced by this strain develops somewhat more rapidly than that induced by the stock strain. The gross lesion at 24 hours is indicated by a grayish white exudate which rapidly progresses into a widely spread, thick moist purulence with only a slight tendency to localize. From 48–72 hours the exudate takes on a grayish ochre color, remains moist and sticky, and may develop a necrotic center. Except in two or three instances where only a slight infection was induced there was little tendency for the membrane to dry and heal with scab-like formation.

The behavior of the organism is comparable to that of the stock strain. It grows rapidly during the first 24 hours on the membrane and if the embryo is not killed many streptococci are phagocytosed by, and undergo degeneration within, mononuclears.

The most pronounced difference between the two strains is the lytic effect which Strain II exerts on the red blood cells and the leukocytes. Even at 24 hours the red blood cells are considerably hemolyzed. As the lesion develops smears show many fragmented nuclei. The behavior of the leukocytes is essentially the same as with the stock strain, *i.e.* appearance of polymorphonuclears followed more rapidly by mononuclears, phagocytosis and degeneration of cocci within the cytoplasm of mononuclears. Active mitotic division of mononuclears and the presence of a good many myelocytes are noticeable in these smears.

In comparison with Strain I the mortality rate is decidedly higher with Strain II and its power to invade the embryo more pronounced. Forty-one embryos were inoculated at different times with this strain. Twenty-two of these were dead at 48 hours. A number of embryos were killed for sections. Only 1 chick actually hatched. Blood cultures from this and from the hearts of 8 live embryos autopsied at different hour intervals were all positive for

*Str. haemolyticus.* Five spleens were cultured and each was positive for *Str. haemolyticus.*

This strain was carried through three generations on the membrane. The filtrate of a 24 hour broth culture was used to inoculate 10 14 day embryos and 2 12 day embryos in amounts varying from 1 capillary drop to 0.5 cc. No gross membranal lesions developed. As judged by smears, leukocytic reaction was questionably stimulated. All these embryos lived to hatch.

*Strains III and IV:* "Todd" and "sputum" strains were obtained from Dr. H. M. Powell, Eli Lilly Laboratory. The virulence of these strains had been raised until one millionth of 1 cc. of a 24 hour broth culture was fatal for mice. Inoculums in various experiments consisted of 1 capillary drop of an undiluted 24 hour broth culture; 1 capillary drop 1:5 dilution 24 hour broth culture; and 1 capillary drop 1:10 dilution.

All embryos inoculated with these strains died within 48 hours. They usually survived 24 hours. The "Todd" strain grew better on the membrane than the "sputum" strain. The latter strain killed the embryos more quickly. In one instance 3 of 5 embryos inoculated with "sputum" strain were dead at 18 hours. The membranes tended to appear bloody. Leukocytes were present in smears.

*Comment:*

Sections of the membrane of resistant embryos show an abundant superficial growth of streptococci forming a more or less continuous sheet or film. This bacterial membrane tends to become surrounded by cellular exudate which fuses to form a hyalinized zone. Thus encapsulated many cocci undergo degeneration. *Str. haemolyticus* tends to cause necrosis of the membrane in the central portion of the inoculated area and the bacteria grow abundantly on the necrotic surface.

There is little or no evidence of direct invasion of the embryonic tissues in sections of infected membranes. That the cocci do gain access to the embryo is evident from positive blood and spleen cultures. Microscopic examination of embryonic organs has shown no evidence of bacterial infection. However, in partially autolysed organs of dead embryos streptococci are often abundant in blood vessels, especially of the hepatic sinuses, representing postmortem growth. The bacteria are extracellular.

Smear preparations show that *Str. haemolyticus* is especially phagocytosed by mononuclear cells rather than by polymorphonuclears. Within the mononuclears they rapidly undergo degenerative changes, becoming very small granules which are eosinophilic rather than basophilic in their staining reaction, and which tend to become Gram-negative. Some of these mononuclear phagocytes containing a group of small forms with a few larger basophilic cocci remind one of the inclusions seen in smears from inclusion blennorrhea.

It seems evident that *Str. haemolyticus* grows by preference on the surface of the ulcerated membrane and on necrotic tissue. There is no evidence that it proliferates within cells of the exudate or in the tissues of the host, although it may remain viable in the body of the living embryo. In these respects it resembles *Staph. aureus*. The leukocytic cytoplasm, especially that of mononuclears, is capable of destroying the cocci.

### *Streptococcus viridans*

Only one strain of this microorganism was used. It was isolated at Vanderbilt Hospital, June, 1935, from the blood stream of a patient with endocarditis. The cocci are small and grow in extremely long chains. The inoculum consisted of 1 capillary drop of a 24 hour broth culture.

The first grossly evident change on the membrane is the appearance of hemorrhage or patches of free blood on the surface. Smears show the presence of leukocytes in this bloody fluid. The organism may grow riotously but it does not grow well on the membrane as consistently as do the hemolytic strains of streptococcus. The gross lesion does not progress to any remarkable degree. The thin bloody exudate dries and becomes closely adherent to the surface of the membrane. Although in several instances the cocci did not appear to grow well, in others there was an abundant growth almost entirely confined to the interior of mononuclear leukocytes. These cells were filled to the point of rupture with well preserved, deep blue staining cocci, which showed little or no evidence of intracellular degeneration.

The mortality rate is low. Pure cultures of streptococcus were obtained from 6 of 7 blood cultures taken from live embryos at autopsy. Only one spleen was cultured and it was negative. Five

hatched chicks were autopsied. The organism was recovered from the blood stream of each.

The strain was carried through four generations on membranes.

*Comment:*

Although relatively few experimental inoculations were performed with *Str. viridans* as compared with *Str. haemolyticus* certain very evident distinctions appear in the infective process. Inoculations with *Str. viridans* do not lead as frequently to extensive lesions on the membrane as do those with *Str. haemolyticus*, nor is the inflammatory reaction so abundant usually. In 2 cases, however, from a limited series in which smears and sections of the membrane were made, a mechanism of invasion was evident which did not appear in any case with four different strains of *Str. haemolyticus*. There was a rich mononuclear exudate on the ulcerated surface of the membrane and smears showed that the cocci were growing abundantly and rapidly within the cytoplasm of these cells, with little or no evidence of degenerative change either of the cells or of the bacteria. The cocci also grew extracellularly in a sheet on the surface, but not so numerously as did *Str. haemolyticus*; and having become encapsulated by hyalinized cells they underwent degenerative changes.

Sections of such an infected membrane show a remarkable invasion of the mesodermal tissue. There is little inflammatory exudate deep in the mesoderm, but beneath and to the side of the surface infection streptococci are growing wildly within the wandering mononuclear cells and in the fixed fibroblasts. There is no certain evidence that growth is taking place in the intercellular tissues. The cytoplasm of macrophages and of fibroblasts is often filled with rounded groups of cocci; and in places where fibroblasts are infected there is no evidence of cellular injury or inflammatory reaction. No cocci are found in ectodermal and entodermal epithelium.

The capacity of growth of this strain of *Str. viridans* within the cytoplasm of mesodermal cells is seen also in sections of the embryonic organs in these cases. Kupffer cells of the liver of an embryo, killed for microscopic study, are filled with cocci; the serosal cells of the peritoneum are solidly packed with them, forming a blue lining to abdominal organs in preparations colored with Wright's stain. Many glomerular tufts of the kidney are distended with

cells containing the microorganisms and the perirenal tissues contain abundant collections of macrophages loaded with them. There is little or no necrosis and no inflammatory reaction.

It is evident that *Str. viridans* under suitable circumstances will invade the tissues of the chick embryo by growing within the cytoplasm of mesodermal cells, including macrophages. It appears from sections of the membrane that extension of infection to deep connective tissue can take place directly by growth from one fibroblast to another.

Phagocytosis, under these circumstances, instead of hindering favors tremendously invasion of the host.

### *Aerobacter aerogenes*

Strain 884, American Type Culture, fecal strain isolated in China, was used for the inoculations.

Primary inoculations on 13 day embryos were made with 2 capillary drops of a turbid water suspension in some instances, and in others with 2 capillary drops of a 24 hour broth culture. The strain was maintained from the second through the eighth generation by loop transfers of material from membrane to membrane at 72–96 hour intervals. Growth of this organism can be easily maintained in this way. The manner of leukocytic reaction is the same as in coccus infections, although the quantitative leukocytic response falls below that to streptococci. Phagocytosis is evident and becomes pronounced when an old culture is used. At the third or fourth transfer the strain becomes established at a rapidly growing vegetative level. Long slender forms as well as coccoid forms then fail to appear.

After inoculation with an active culture the membrane becomes cloudy with bacterial growth. On a moist membrane the fluid becomes turbid. There are leukocytes present. After a small inoculation or an inoculation with an old culture the leukocytic reaction is rapid and abundant. Practically all the organisms are phagocytosed and a formidable localized exudative lesion develops.

Approximately 50 membranes were inoculated with *A. aerogenes*. No embryos died before 36 hours. The highest mortality was between 48–72 hours. Twenty to 25 per cent survive 72 hours and one may expect a very small percentage to hatch.

A strain of *A. aerogenes* isolated from a case of granuloma inguinale behaved through two generations in the same manner as the stock strain.

*Smears and Microscopic sections:* There is massive bacterial growth on the surface of the lesion, and necrosis as well as ulceration of the membrane frequently occurs at the center. Beneath the bacterial layer is a zone of cellular exudate which in turn is walled off from the deeper tissues by a layer of giant cells.

Smears from the exudate in the early stages of infection show an abundance of intracellular bacilli, especially in mononuclears. While some variations in form of the incorporated bacteria appear, it seems evident from the number of microorganisms present and the absence of evidences of degeneration that these bacilli are capable of energetic growth within the cytoplasm of these cells. This judgment is supported by microscopic sections, not only of the membranes but of internal organs of the embryo as well. In 1 membrane the bacilli have undermined the entodermal epithelium and have invaded and grown profusely within the cytoplasm of these cells. In another embryo there was a focal necrosis of the brain in which mononuclear cells were filled with bacilli evidently growing there. In this case also there was an extensive infection of the lung with profuse growth of bacilli within epithelial cells of pulmonary alveoli and bronchioles.

The cells in which bacilli are incorporated and growing usually show little evidence of damage except changes in form due to the volume of bacteria and associated vacuolation. There is little necrosis and practically no inflammation.

*Comment:*

*A. aerogenes* grows profusely on the membrane extracellularly causing ulceration and necrosis. In the deeper tissues and internal organs evidences of growth are found only within the cytoplasm of cells, the mononuclear leukocytes and entodermal epithelium. It seems obvious in these cases that phagocytosis by mononuclear leukocytes favors rather than retards growth and invasion. Internally, continuation of growth and invasion seem to depend entirely on incorporation of the microorganism by cells, especially those of entodermal epithelium.

*Eberthella typhi*

A stock strain isolated at Vanderbilt University in 1934 was used for inoculation. The inoculums in various experiments consisted of 1 capillary drop of 24 hour broth culture, 1 loop of 24 hour broth culture, and 1 capillary drop of 24 hour broth culture diluted 2:3 with sterile NaCl solution.

An inoculation of 1 capillary drop of 24 hour broth culture produces a condition in which the bacilli grow riotously. Massive growth of the organism is visible grossly. There is very slight leukocytic reaction and rapid mortality. A series of 14 embryos thus inoculated gave a death rate of 4 at 18 hours, 4 others at 48 hours, and all before 72 hours. Another series gave some survivals through 96 hours.

Embryos inoculated with 1 loop of a 24 hour broth culture permit only a very slight growth of the organism with an abundant leukocytic reaction (polymorphonuclears followed by mononuclears). One smear in which the microorganism was lost sight of showed a moderate mononuclear reaction at 96 hours. Of 6 thus inoculated, 2 lived to the day of hatching; 2 others were killed for histological sections. The same reaction and mortality rate occurred when an old culture was used for inoculation. One of these chicks hatched.

The most pronounced lesions were produced by the inoculation of 1 capillary drop of 2:3 dilution of 24 hour broth culture. Only 1 of 18 was dead at 24 hours. Blood vessels were thrombosed and there was extensive growth of the microorganism. At 72 hours 8 of the 18 were alive. The lesions were expansive. Several were extensively necrotic and of a muddy color. There were many bacilli and necrotic cells in smears. A few lesions tended to localize without much necrosis. These localized lesions were grossly comparable to those of *A. aerogenes*. Of 18 embryos, 3 lived to the 5th day and 1 to the 6th day. Several were killed for sections.

Material from this series was transferred to other eggs and the bacillus was maintained with ease through four generations. Bacilli grew rapidly at each transfer and it is concluded that the strain could be carried on indefinitely from membrane to membrane.

Blood cultures were taken at four autopsies. Each was positive for Gram-negative bacilli, as were 3 cultures made from one gallbladder.

On the whole the hearts at autopsy were pale and flabby. A few of the spleens were enlarged. The kidneys were swollen. One autopsy revealed a milky pericardial fluid, and smears showed lymphocytic and monocytic exudate.

*Microscopic Sections and Smears: E. typhi* grows rapidly and abundantly on the inoculated membrane causing ulceration and usually an extensive necrosis of the central portion of the lesion. Its growth is more injurious to the membrane than that of any other bacterium studied. Internal organs show evidence of damage, especially the kidneys, which are grossly swollen and show cloudy swelling and colloid degeneration of tubular epithelium microscopically.

In sections as well as smears there is some polymorphonuclear response early, but mononuclears are usually predominant. Within the mesoderm of the membrane, both fixed fibroblasts and mononuclear cells are extensively vacuolated, irregular in outline and disoriented. This suggests injury by soluble poisons.

There is very little phagocytosis of these microorganisms evident either in smears or sections. The leukocytes seem to be injured. There is no evidence that *E. typhi* grows within the cytoplasm of the cells of the exudate. Mesoderm of the membrane shows no direct invasion by bacilli except in areas of necrosis where the bacteria grow abundantly.

It is of great interest to observe, however, in 3 cases out of 5 which were studied histologically that entodermal epithelium becomes focally invaded by the bacilli; and under these circumstances irregularly rounded intracytoplasmic masses of these organisms appear surrounded by a halo. The intracellular bacilli seem to stick together, although the individual forms can be resolved under the microscope. When the growth reaches a certain size the cell becomes vacuolated as if it could easily rupture. These focal areas may be quite extensive and they are not associated with evident extracellular bacterial growth in the neighborhood of the foci of infection. These intracellular masses of bacilli evidently represent intracytoplasmic growth of *E. typhi* in this tissue, although the mechanism of entrance into the cells does not appear. The incorporated bacilli are much smaller than those growing extracellularly on the ulcerated or necrotic surfaces. In one instance a focus of similar infection in ectodermal epithelium was found.

Examination of internal organs and tissues of several embryos has shown no evidence of bacterial growth, although cultures from blood and bile have yielded positive growth.

*Comment:*

No other bacterium studied has exhibited this type of cellular invasion and growth. Entodermal epithelium appears to be especially susceptible and this constitutes a possible intracellular medium for growth, protection and extension of the infecting bacillus.

One may judge from the appearance of the microscopic picture that growth of *E. typhi* on the membrane is associated with the absorption of potent injurious substances. It is of interest that the rate of the heart beat of the infected embryo decreases, and the myocardium becomes quite pale. Degenerative changes in parenchymatous organs are likewise indicative of severe intoxication.

### *Brucella (alkaligenes) abortus*

This culture of *Br. abortus* came originally from the Hygienic Laboratory, Washington, D. C. The artificial medium used for culturing the bacilli has been a Russell's agar slant without an indicator.

Six 14 day embryos were inoculated with 1 capillary drop of a turbid saline suspension of a 48 hour slant culture. There was very little gross lesion noticeable after 24 hours. A smear at this interval gave little evidence of bacterial growth. At 96 hours the membranes were thickened and extensively cloudy. With the exception of one small focus on 1 membrane there was no noticeable exudative reaction. A smear at 96 hours showed intracellular growth of the organism within ectodermal epithelium. One embryo bearing the localized focus of exudate lived into the 7th day. The amount of exudate increased slightly. The reaction was almost completely a mononuclear one.

A second generation of the bacillus on membranes was initiated by inoculation with bits of tissue from a live membrane at the 96 hour stage. At 48 hours the lesions of the second generation were more localized and more exudative than the first generation lesions. The exudate was moist, sticky and mononuclear. Very few organ-

isms were found in smears. One embryo was dead at 48 hours; another was dead at 4 days; and 1 lived to the day of hatching.

Another second generation series of 6 embryos received 1 and 2 capillary drops of a suspension of organisms cultured on a slant from a first generation membrane. The 48 hour lesions were moist, radiating and exudative. Smears showed bacterial growth outside as well as inside the cells. All embryos were dead at 5 days.

*Comment:*

Infection of the chick membrane with *Br. abortus* leads to relatively little inflammatory exudation. The bacilli enter ectodermal epithelial cells and proliferate there, as they do in chorionic epithelium of the calf.[3] If a cellular exudate occurs it is predominantly a mononuclear one, and the bacilli after entering these may grow abundantly.

*Br. abortus* penetrates the membrane, and a very characteristic lesion in microscopic sections is the presence of small nodules consisting of proliferated fibroblasts. In these cells it is possible to demonstrate in some preparations the presence of bacilli. The bacteria do not apparently gain entrance to entodermal epithelium, and no lesions or microorganisms have been found in internal organs.

In the dead embryo, and in case an area of necrosis of the membrane is present, *Br. abortus* grows abundantly extracellularly in the neighborhood of dead cells.

From these observations it appears that *Br. abortus* invades the host by virtue of its ability to maintain viability and to multiply within the cytoplasm of cells. Both ectodermal epithelium and mesodermal cells (large mononuclear cells and fibroblasts) serve as suitable media for invasion and propagation.

### *Mycobacterium tuberculosis avium*

Our strain was isolated in this laboratory Jan. 24, 1934, from the liver of a fowl brought in for examination. A very turbid suspension of growth from a Petragagni slant was inoculated in 1 capillary drop amounts onto membranes of 6 and 8 day embryos. One series of 14 day embryos received 2 capillary drops.

Six day embryos did not survive for more than 4 days. There is no noticeable gross lesion at 24 hrs. At 48 hours membranes appear

thickened, clear and gelatinous. Throughout their development the lesions are noticeably in, rather than on, the membranes. After 4–5 days the membranes are dry with a few nodular foci. After 96 hours it is difficult to get material, either cells or bacilli, for a satisfactory smear. At 24 hours there is an increase in polymorphonuclears and a few mononuclears. Both kinds of leukocytes show phagocytosis. At 48 hours there is a decided increase in the number of mononuclears and probably giant cell formation. There is phagocytosis of polymorphonuclears containing acid-fast bacilli by clasmatocytes. Smears demonstrate an increased number of bacilli within phagocytes through 72 hours.

Loop transfers to another membrane failed to produce a grossly visible infection. Other methods of transfer were not tried.

The mortality of embryos is negligible. Almost all the embryos live until they are due to hatch. During the course of observation the embryos are strong and active. A number of embryos that pipped the shells were unable to hatch completely. Three chicks hatched, 1 of which died soon afterward. Two were observed for several days. The chicks were weak from the beginning. They continued to weaken to prostration. One was autopsied 3 days after hatching. Smears from a peritoneal fluid showed acid-fast bacilli, but contamination was too great for culture. Another chick was autopsied 5 days after hatching. No abnormal peritoneal fluid was found and smears were negative.

The organism was never recovered from spleens or livers. No gross lesions appeared in the internal organs.

*Comment:*

*Myco. tuberculosis avium* gains access readily to the mesodermal cells of the inoculated membrane. There is very little inflammatory exudate on the surface, but sections stained to demonstrate acid-fast bacteria show multiple foci within the mesodermal tissue composed of large mononuclear cells, many of which are loaded with bacilli; there is a proliferation of fibroblasts and these cells likewise contain within their cytoplasm varying numbers of bacilli. No extracellular groups of bacilli are evident.

The interesting feature of this dissemination of acid-fast bacilli is the fact that cells having all the characteristics of fibroblasts and far removed from the larger foci of bacilli contain within their cyto-

plasm considerable numbers of the microorganism. This dissemination simulates that which occurs in infection of the membrane by *Str. viridans* and *Br. abortus*, although there is no indubitable evidence of growth.

The short period of observation allowed by the incubating eggs does not permit proof of intracellular growth of *Myco. tuberculosis*, although the evidence indicates that these microorganisms have multiplied within the large mononuclears. Invasion is certainly through intracellular channels.

The bacilli may gain entrance to the interior of the embryo, even within a short time; for a few microorganisms were found in sections from 1 case in Kupffer cells of the liver.

### *Corynebacterium diphtheriae*

*Strain I:* Isolated from the heart's blood of a case of acute vegetative mitral endocarditis (*C. diphtheriae*).[4]

Primary culture of the organism in broth was granular. The granules settled to the bottom of the tube leaving a clear supernatant broth. The organisms were short, irregularly shaped bacilli. Often very short rods would be rounded at one end and pointed at the other. Cytoplasm of the bacilli tended to stain diffusely. Inoculation of this short irregular bacillus to membranes gave in 24 hours long slender, club shaped rods with metachromatic granules. The embryos were noticeably sick at 24 hours and all dead before 48 hours. By careful rapid transfers on proteose peptone broth the organism was easily induced to grow on the surface of liquid medium with the formation of a very heavy pellicle. For inoculums in various experiments the following were used: 1 capillary drop of 24 hour broth culture; 1 capillary drop of clear broth drawn from beneath the pellicle of growth; small amounts of growth taken from the surface of pellicle; and 1 capillary drop of 1:200 dilution of shaken broth culture. In every case the embryos were dead before 48 hours and in only a few cases did embryos live past 24 hours.

The bacilli grow abundantly on the surface of the membranes in colony formation. Plaques of growth are rounded, flat, overlapping, and noticeably not adherent to the membranes. There is massive growth of the organism with very little leukocytic reaction.

Transfers of the organisms from membrane to membrane at 24

hour intervals were made through ten generations. The morphology and character of the growth remained unchanged. Change in pathogenicity was not detected. During this experiment most of the transfers were made from dead membranes. The amount of growth on 2 live embryos in two different series which were observed at 12 hour intervals showed that the organisms had grown abundantly in that length of time on live membranes. The growth used at 24 hour intervals from dead membranes must not have represented a decided proportion of postmortem growth. Toxin was produced from this tenth passage strain of the bacillus.

Two live embryos were autopsied 12 and 14 hours after inoculation. The membranes bore the characteristic massive growth of bacteria. The whole embryo — muscle, stomach, spleen, liver, and so on — was hemorrhagic.

A toxin was prepared by growing the bacilli on the surface of a flask of proteose peptone broth for 7 days, then centrifuging and filtering the broth through a Berkefeld V candle. This toxin was inoculated onto embryonic membranes in amounts of 1, 2, 3 and 4 capillary drops. At 24 and 48 hours there was considerable congestion and thrombosis of membranal blood vessels with petechial hemorrhage. Leukocytic reaction was not noticeable grossly but smears revealed a good number of polymorphonuclears which were in turn followed by monocytes. Embryos remained strong. Most of them lived to hatching. Four drops of toxin were not a lethal dose. The only grossly apparent embryonic lesion, except the membranal one, was a greatly enlarged, pale waxy spleen.

A $\frac{1}{2}$ capillary drop of diphtheria antitoxin put onto membranes 24 hours previous to inoculation protected the embryos against 2 capillary drops of a 24 hour broth culture. No attempt to determine a minimum protective dose of antitoxin was made. One drop of antitoxin followed in 24 hours by 1 drop of culture produced very little membranal reaction. There was no flaky bacterial growth at all. The membrane tended to be dry. Bacilli were present in smears but they were short and pale. A few localized foci of leukocytes increased slightly in size from day to day. At 72 hours the organisms looked more active. At 96 hours there was evident growth. A good many were slender forms with metachromatic granules. This increased growth of the organism did not kill the embryos. A large percentage of embryos protected by antitoxin was able to hatch.

With a decreased dose of antitoxin and an increased amount of culture a large leukocytic lesion, mounded, dry and localized may be induced. Under this circumstance the bacilli are not as definitely inhibited. The lag period is shorter but the embryo is still protected.

A strain of the organism has been kept in the presence of antitoxin on membranes for 6 weeks. With the exception of two short intervals it has been on no other medium. Between the first and second transfers a plate culture was made on blood agar. Between the eighth and ninth transfers a blood agar culture intervened. Other transfers were made either by loop or by clipping a small piece of infected material and rubbing it over the surface of the membrane to establish the next generation. Protection by passive immunity throughout this experiment was perfect. Ten serial transfers were made at from 4–6 day intervals.

*Strain II:* A strain of Park-Williams No. 8 diphtheria bacillus was inoculated onto membranes in capillary drop amounts. Aside from some vascular congestion comparable to that produced by pure toxin there was no gross membranal lesion at 24 hours. Smears revealed a number of leukocytes and red blood cells. No organisms were found. The embryos were all dead at 48 hours. Postmortem growth of the bacilli is questionable. Inoculation of a membrane with an autogenous toxin or with an antitoxin 24 hours previous to addition of the culture did not promote growth of the organisms. Antitoxin protected the embryos from death. Very heavy inoculations of growth from a Loeffler's slant to membranes produced death in less than 24 hours. In these cases there was what appeared to be postmortem growth of the bacillus. One embryo which lived beyond 24 hours showed no organisms on smear. Transfer of material from a dead embryo (heavy inoculation) to a second generation gave the same results. Two other like transfers from dead embryos were made. In the fourth generation 1 live embryo was found which appeared to support growth of the bacilli. Transfers were made from this egg to establish a fifth generation where the strain was completely lost.

A stock strain of *C. hofmanni* did not grow after inoculations from broth or from slant cultures. There was evidence of postmortem growth on 1 membrane. This strain did not kill the embryos.

Two hundred embryos were used with this diphtheria-diphtheriod work.

*Comment:*

A freshly isolated strain of *C. diphtheriae*, a moderate toxin producer, grows abundantly on the chorio-allantoic membrane, but an old laboratory strain, a strong toxin producer, apparently does not grow at all or very little, although it kills the embryo quite rapidly.

The freshly isolated strain grows as flaky colonies at the surface of the membrane apparently not attached to it. There is no inflammatory reaction, but injury to the blood vessels resulting in dilatation, stagnation and hemorrhage soon makes its appearance. There is no necrosis of the superficial epithelium. No phagocytosis or invasion of the tissues is evident.

Conditions are altered however by the administration of antitoxin 24 hours before the inoculation. Under these circumstances the bacilli grow less readily and there results an abundant cellular exudation, as well as focal necrosis of the membrane. The embryo is protected by the antitoxin and survives the infection. Protection of the embryo from the effects of diphtheria toxin by the administration of antitoxin was demonstrated recently by Ozawa,[5] using our chick embryo technique.

The recently isolated strain does not tend to invade the tissues of the membrane, but grows abundantly on the surface of necrotic tissue even in the antitoxin-protected embryo.

## Discussion

Ever since substantial foundations for the science of immunology were laid, investigations into the phenomena of infection have taken in the main two directions, namely those that concerned serum antibodies and those that endeavored to disclose the rôle of phagocytosis.

The proved and implied potentialities of these two possible mechanisms of defense against bacterial infection seem to have dimmed the vision of both pathologists and bacteriologists to the basic problems which are the counterpart of resistance, namely susceptibility.

Although natural resistance to infection is of great general biological interest, it would seem that an explanation of natural susceptibility would be essential for a proper understanding of that phenomenon; yet one looks in vain for stimulating hypotheses in

this territory comparable to those of serum antibodies and phagocytosis in the adjoining field of resistance. The fact that the latter do not explain satisfactorily acquired immunity to bacterial infection may in part be due to a lack of knowledge of susceptibility.

Metchnikoff, actively championing the cause of positive defense by means of phagocytosis, viewed susceptibility negatively as a breakdown of this mechanism through the repulsion of germ-eating cells. The serologists were often at a loss for a reasonable explanation of susceptibility for they soon learned of instances in which the serum of susceptible hosts was bacteriolytic for the infectious agent, and of others in which despite the absence of demonstrable antagonism of the body fluids natural immunity to the microbe was complete. An understanding of susceptibility involves a knowledge of the host-parasite relation in the early stages of invasion, and this knowledge of most infectious diseases is very meager.

Before the theory of phagocytosis as a defensive mechanism had gained so universal an acceptance as it now enjoys there were those who presumed to regard the intracellular medium, even of leukocytes, as a favorable menstruum for the growth of certain bacteria during the period of invasion, but Metchnikoff thought that this could happen only if the phagocytes were severely injured or dead.

Little was said by the Metchnikoff school about those protozoal diseases in which the parasite obviously undergoes its growth and metamorphosis within the protoplasm of cells; and no acceptable lodging place of hypothesis has even yet been found for the invasive phenomena of a group of infectious agents constantly gaining in recognition and including not only protozoal but bacterial, fungal, spirochetal, rickettsial and viral forms, which seem to defy the selective destructive forces of cytoplasm, whether of mobile phagocytes or of tissues that are fixed.

One of us has recently called attention to this group of infectious agents, tentatively recognizing extracellular, facultative intracellular and obligate intracellular parasites.[6]

In the experimental study here presented it has been found that the embryonic tissues of the developing chick offer a relatively simple and uniform medium in which some of the phenomena of invasion and susceptibility may be observed. It is probable that a large proportion of pathogenic bacteria in pure culture will induce infective lesions in the chorio-allantoic membrane. Such lesions may be

observed at will; while smears, cultures and sections of tissue may be obtained at desired intervals.

The limited number of pathogenic bacteria thus far studied in this preliminary survey of the method have shown that infective lesions may be induced with cultures of *Staph. aureus*, *Str. haemolyticus*, *Str. viridans*, and *A. aerogenes*, *E. typhi*, *Br. abortus*, *C. diphtheriae*, and *Myco. tuberculosis avium*. Of this group all but *Staph. aureus*, *Str. haemolyticus* and *C. diphtheriae* invade the cells in and about the lesion and find within either mobile or fixed cells, or both, a favorable intracytoplasmic medium for maintenance and growth. Different cells of the host vary in their availability for intracellular growth of these parasites. *Str. viridans* was found intracellularly only within wandering and fixed mesodermal cells; *A. aerogenes* in wandering mesodermal cells and in entodermal epithelium of the chorio-allantois and lung; *E. typhi* in entodermal epithelium of the membrane, and to a slight extent in membranal ectodermal epithelium; *Br. abortus* in ectodermal epithelium and mesodermal elements of the membrane; and *Myco. tuberculosis avium* in mesodermal elements.

While this embryonic medium may not be comparable with the natural hosts for the bacteria studied, it is of interest to note that the experimental lesions in the membranes are characteristic for the bacterial types and in general simulate those found in the natural host. This is significant. So little is known about the early stages of natural infection, the stage of invasion and so-called period of incubation, that one may well obtain working hypotheses from membranal infections which would be helpful in restudying the more complicated phenomena in the natural host.

One may draw the conclusion, at least tentatively, that the cells of susceptible hosts not infrequently present themselves as available media for the growth of infectious agents before general immunity manifests itself. Judging from the experiments presented the phenomena of phagocytosis seem to be a greater aid than impediment to invasion by many kinds of pathogenic bacteria.

The method which we have used would seem to have useful applicability to the study of problems of immunity as well as of susceptibility, for it is quite evident from the results with diphtheria antitoxin that serum antibodies may be effectively introduced into the embryo in quantity sufficient to immunize it against otherwise

fatal doses of toxin. The problem of the effect of antitoxin on the *in vivo* growth of *C. diphtheriae* is also rendered approachable. In like manner it is possible that bacterial antibodies and complement itself may be studied individually or together in such a medium; and their behavior in the presence of specific bacterial infection may be thus exposed to analysis more effectively than is now easily possible.

While an antagonism between phagocytes and certain bacteria is well recognized, it seems probable that in a large proportion of infectious diseases the invading agent finds a favorable lodging place within cells during the stage of invasion and through them grows and extends.

Phagocytosis therefore is not synonymous with host resistance, and it may have two diametrically opposed effects, namely inhibition and facilitation of the infectious process. So far as phagocytosis is related to resistance, the effect of the intracellular environment on the invading bacteria is the determining factor.

How non-motile and motile bacteria gain admission into fixed cells of mesodermal, ectodermal and entradermal origin is an important problem. Some of our observations indicate the possibility of invasion from one cell to another by growth through contiguous cytoplasmic processes.

## Conclusions

1. Inoculation of the chorio-allantoic membrane of chick embryos with pure cultures of pathogenic bacteria is a practical method for studying many problems of infection, especially the early stages of invasion.

2. It is indicated in this survey of the method of studying bacterial infection in chick embryos that many pathogenic bacteria find in either mesodermal cells (fixed or mobile) or epithelial cells, or both, favorable and possibly necessary media for invasion of the living host.

3. In these instances phagocytosis instead of representing resistance to infection actually favors it.

4. Among those pathogenic bacteria that are able to utilize a living intracellular environment for growth are *Str. viridans*, *A. aerogenes*, *E. typhi*, *Br. abortus* and *Myco. tuberculosis avium*.

5. *Staph. aureus* and *Str. haemolyticus* may be in part destroyed by the phagocytes of the embryo, and they appear to be incapable of growing in an intracellular medium in this host.

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## DESCRIPTION OF PLATES

### PLATE 20

FIG. 1. *Str. haemolyticus*; 72 hour lesion; purulent exudate.

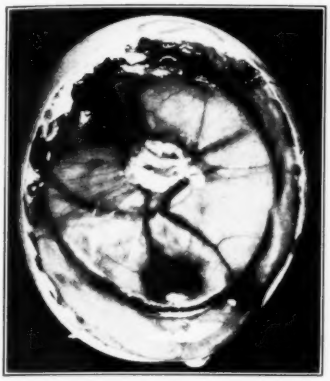
FIG. 2. *A. aerogenes*; 72 hour lesion; bacilli and exudate.

FIG. 3. *E. typhi*; 5 day lesion; bacilli and exudate.

FIG. 4. *C. diphtheriae*; 24 hour growth; flakes of bacilli.

FIG. 5. Diphtheriae antitoxin; 24 hours. *C. diphtheriae*; 72 hour lesion; purulent exudate.

FIG. 6. Diphtheria toxin; 48 hours. Petechial hemorrhages.



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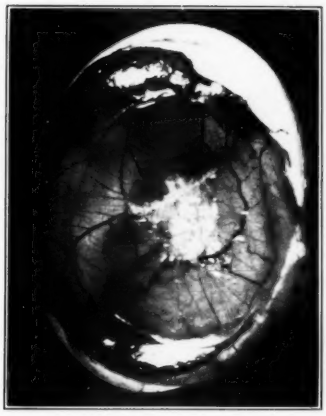

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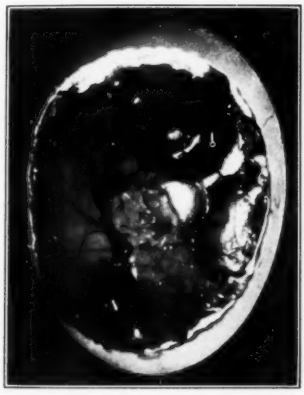

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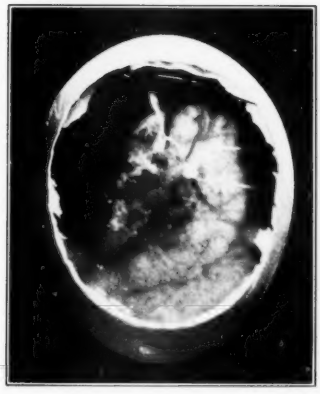

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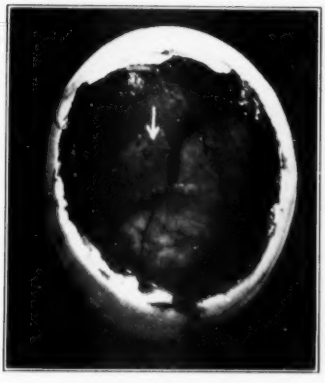


6

Goodpasture and Anderson Bacterial Invasion of Chorio-allantoic Membrane

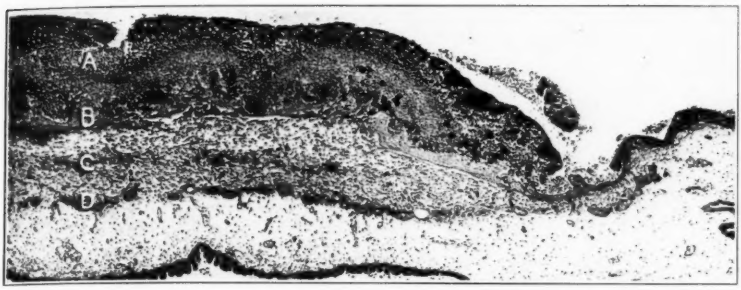
PLATE 21

FIG. 7. *Staph. aureus*; membranal lesion; 72 hours. × 46.
(*a*) Leukocytic exudate containing dark clumps of cocci.
(*b*) Giant cell membrane.
(*c*) Granulation tissue.
(*d*) Remnant of ectodermal epithelial layer.

FIG. 8. *Str. haemolyticus*; membranal lesion. Ulceration, exudate and necrosis (right). × 46.

FIG. 9. *Br. abortus*; membranal lesion; 96 hours. Little exudate, no ulceration, deeply seated fibroblastic nodules. × 46.

FIG. 10. *E. typhi*; membranal lesion; 48 hours. Surface bacterial growth (upper layer). Arrows point to intracellular masses of bacilli in entodermal epithelium. × 250.

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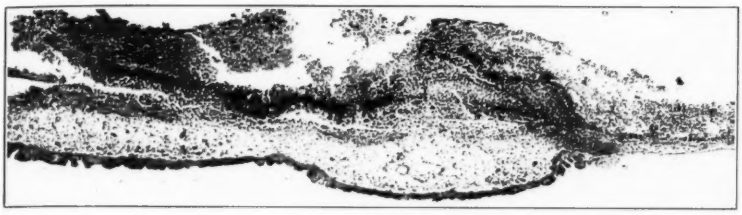

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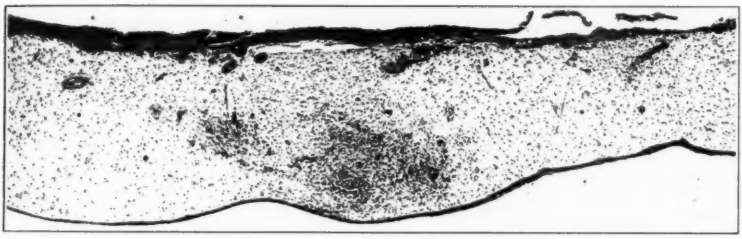

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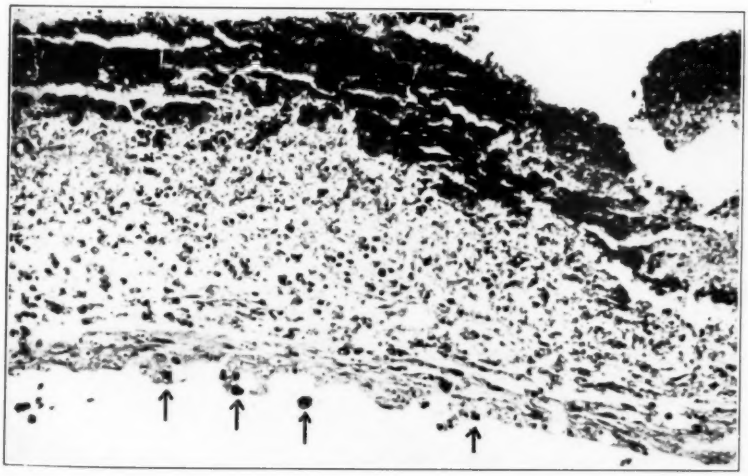

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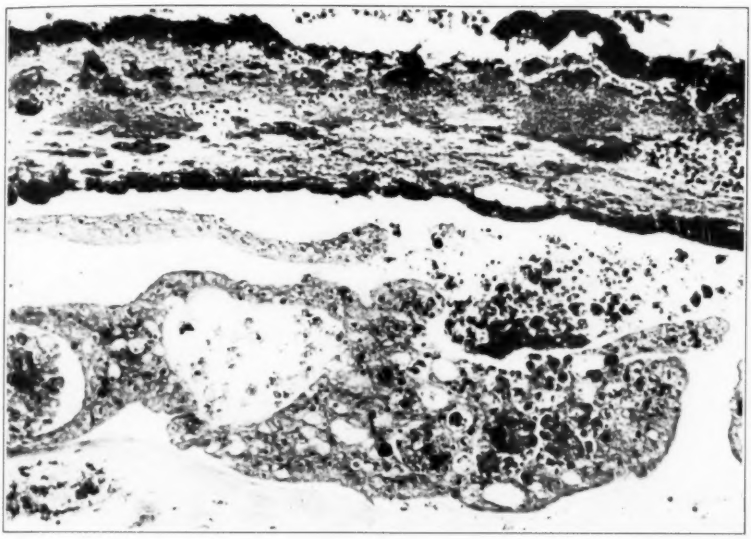
Goodpasture and Anderson Bacterial Invasion of Chorio-allantoic Membrane

PLATE 22

FIG. 11. *A. aerogenes*; membranal lesion; 96 hours. Dark layers are bacilli. Below is hyperplastic entodermal epithelium containing intracellular masses of bacilli. × 250.

FIG. 12. *A. aerogenes*; lung lesion; 96 hours. Same embryo as Fig. 11. All dark areas represent intracellular groups of bacilli in pulmonary epithelium. All bacilli are intracellular. × 250.



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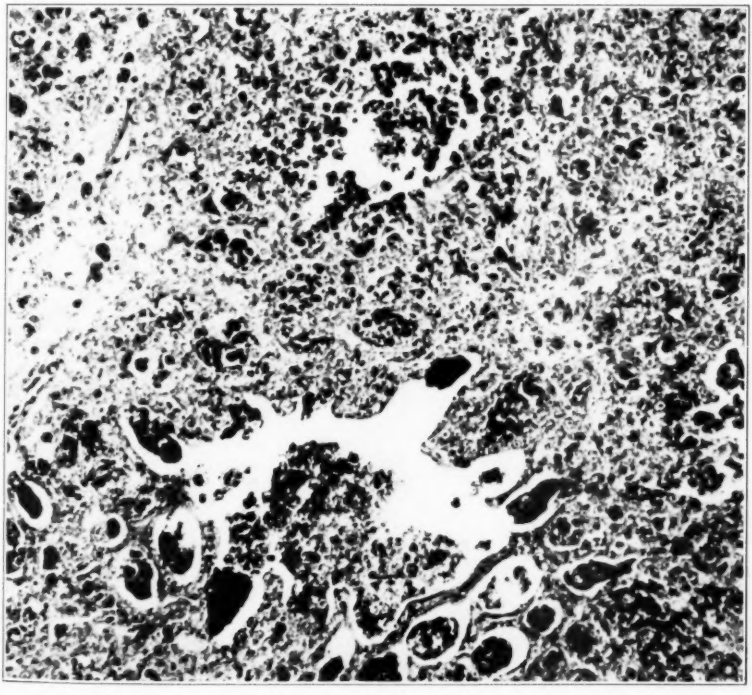

12

Goodpasture and Anderson Bacterial Invasion of Chorio-allantoic Membrane

PLATE 23

FIG. 13. *Str. viridans*; membranal lesion; 72 hours. Deeply stained groups of bacilli in fibroblasts of mesodermal layer. × 1000.

FIG. 14. *E. typhi*; membranal lesion; 72 hours. Arrows point to intracellular groups of bacilli in entodermal epithelium. Dark material represents secretion of superficial epithelium. Note absence of neighboring extracellular bacterial growths. × 1000.

FIG. 15. *Br. abortus*; membranal lesion; 96 hours. Intracellular bacilli in ectodermal cells. × 2300.

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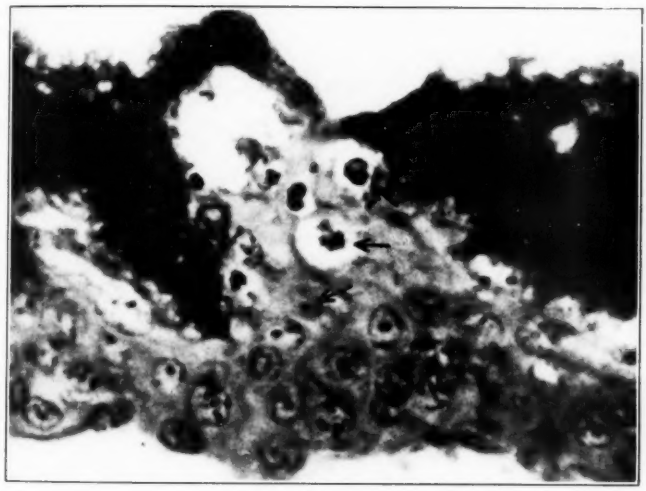

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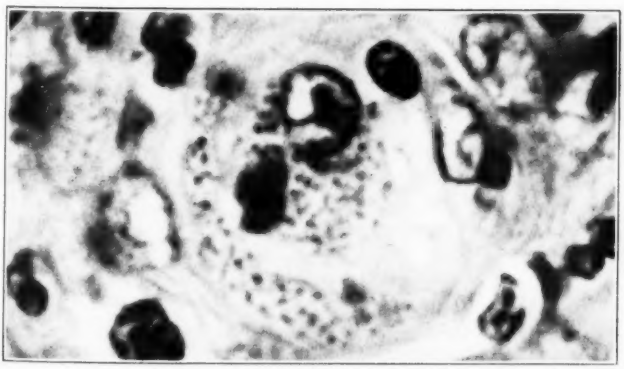

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Goodpasture and Anderson Bacterial Invasion of Chorio-allantoic Membrane

# CONCERNING THE PATHOGENESIS OF TYPHOID FEVER*

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The earliest phases of an infection, involving a portal of entry, a mechanism of invasion, and activities transpiring during the period of incubation, while of the utmost importance to an understanding of the disease as a whole, are the most difficult incidents to discover and are among the least well understood phenomena of most infectious processes.

Invasion of the host and, presumably, multiplication of the parasite, take place before there are manifest evidences of disease. The lesions which follow may at times obscure the pathways of the more cryptic progress of the earlier stages; and in experimental animals it may be difficult or impossible to reproduce conditions comparable to those existent in the original host naturally infected. Even though satisfactory conditions can be found there are technical difficulties that attend a study of these earliest stages of infection, hindering an exposition of them.

Typhoid fever offers a familiar illustration. Despite extensive knowledge of etiology, epidemiology, pathological anatomy and immunology of typhoid fever there is a broad hiatus between the entrance of the specific bacilli into the mouth and the development of manifest symptoms of infection, which is almost entirely unknown terrain. It is not known just where or how the bacilli invade the host and multiply during the incubation period. There are two main hypotheses.[1]

The hematogenic hypothesis supposes that the bacilli penetrate in an unknown way the mucosa of the throat, tonsils or gastrointestinal tract under natural conditions of infection, and on gaining entrance into the blood multiply in this medium and thereby are disseminated throughout the body to localize in the lymphoid tissue of the intestine and other places to induce the specific lesions of the disease as secondary phenomena. The enterogenous hypothesis

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assumes a penetration of the intestinal epithelial lining at some point or points unknown, possibly first that of the Peyer's patches in the lower ileum, followed by a local growth of the bacilli with the production of lesions and a secondary invasion of the blood stream passively through lymphatics and blood vessels. According to this assumption it would seem likely that the original foci of infection would be few and focal, followed by more extensive invasion of the intestinal mucosa after the bacilli begin to be excreted through the bile.

There seems to be no evidence that *E. typhi* actually multiplies within the lumen of the gastro-intestinal tract itself; and its demonstrable presence there is now assumed to be entirely a result of elimination through the biliary tracts.[2]

It has been surprisingly difficult to demonstrate by histological methods any exact relation between the lesions of typhoid fever, whether in the intestine or elsewhere, and the presence of the specific microorganisms. Small groups of bacilli, presumably *E. typhi*, have often been demonstrated in these lesions but they have not appeared to be definitely related to them; and not infrequently it is a question whether or not they represent postmortem growths. It seems to be accepted generally that the earlier the stage of the disease and the sooner after death the tissues are fixed, the more difficult it is to demonstrate bacilli. Mallory states in this connection: "Occasionally the characteristic colonies were found in the mesenteric lymph nodes and in the spleen, but not in any case that came to post-mortem examination very soon after death. In the earliest case (10 days after onset), in which postmortem examination was made one hour after death, no bacteria were found microscopically except along the edges of the beginning ulcerations of the intestine, and in that situation it is of course impossible to differentiate the typhoid from the colon bacillus. Certain it is that when typical colonies of the typhoid bacillus are found in the different organs they bear no intimate relation to the lesions present."[3] Although the typhoid bacillus may not be seen in the lesions their presence is usually demonstrable, by cultural methods, in the intestine, lymph nodes, spleen and bone marrow. It thus appears that the exact portal of entry and the primary site and mechanism of infection in typhoid fever are quite unknown.

Recent studies of bacterial infection of the chorio-allantoic mem-

brane of chick embryos in this laboratory have shown that *E. typhi* grows abundantly on the surface of the membrane at the site of inoculation in association with injured and necrotic membranal tissue and cellular exudate.[4] Furthermore, it was observed that this microorganism is capable of gaining admission into the cytoplasm of living entodermal epithelial cells of the membrane; and under these conditions it apparently will grow intracellularly, forming small intracytoplasmic colonies. The bacteria multiplying in association with necrotic tissue on the surface are quite large, resembling the large forms cultivable on dead media. Those within the entodermal epithelium are on the contrary relatively small and seem to be embedded in a matrix possibly of capsular material. The intracytoplasmic colonies in entodermal epithelium are the only morphological evidence of invasion and multiplication of the bacilli within the tissues of this host, although it was possible on several occasions to obtain positive blood and bile cultures.

This observation of the penetration of the lining entodermal epithelial cells by *E. typhi* and its seeming growth within this living medium suggested the possibility that a similar mechanism might be involved in the invasion of the human intestine by the bacillus, and was the occasion of a restudy of material from several cases of typhoid fever at our disposal.

Particularly suitable for such a study were the tissues from a recent case where death occurred on the 11th day of the disease. A postmortem examination was performed $1\frac{1}{2}$ hours after death. The tissues were fixed immediately in Zenker's fluid, and paraffin sections were stained by immersing them for about 4 hours in Wright's stain (60 drops suspended in 100 cc. of distilled water). The stained sections were differentiated in absolute ethyl alcohol, cleared in xylol and mounted in cedar oil.

## Report of Case

*Clinical History:* A 19 year old white male (V–34–147) was admitted to the Vanderbilt Hospital on Sept. 8, 1934. He had never had typhoid fever nor had he ever received antityphoid vaccine. He began complaining of general malaise 10 days before admission. Two days later after the onset of illness he had a mild chill and felt feverish. He was in bed for 6 days prior to admission and for 3 days vomited everything taken by mouth. At times he was delirious.

On physical examination the spleen was palpable. The temperature was 104.2° F., the pulse 110, and the blood pressure 130/70. Coupled pulse was

noted. The urine contained 2+ albumin with granular casts. White blood cells numbered 4350. The stools were tarry. Guiac test +. Blood culture showed 60 colonies *E. typhi* per cc. The stools and urine were negative for *E. typhi*. The Widal was 1:160.

In spite of all therapeutic efforts his temperature rose to 106° F. He became more restless, requiring restraint, developed signs of peripheral circulatory collapse with severe toxemia, and died on Sept. 9, 1934.

## The Intestinal Lesion and Intracellular Parasitism

Autopsy was performed 1½ hours postmortem. Few of the solitary follicles and Peyer's patches in the intestine showed any gross evidence of ulceration, although all or most of them were considerably swollen.

Microscopically the intestinal lesions are typically those of early typhoid. Frequently a thin epithelial sheet completely covers a patch of Peyer without ulceration. The follicles themselves are swollen by edema and infiltration by numbers of the large mononuclear phagocytes so typical of the cellular response to this infection. There are also areas of necrosis, deposits of fibrin and cellular thrombi in dilated lymphatics and blood vessels. But throughout the agminated follicles there are persistent foci of lymphocytes, plasma cells and reticulo-endothelium which seem to represent original lymphoid nodules. These groups are still fairly abundant although many lymphocytes have been and are being removed by phagocytosis.

First a careful study was made of glandular and surface epithelium, both that covering the lymphoid nodules and that of intervening areas, to see if there were evidence of intracellular bacillary forms such as were found in the entoderm of the embryonic membranes of the chick. No such forms have as yet been found in sections of stomach, duodenum, jejunum, ileum and colon. Furthermore, in accordance with observations of others, no bacteria were found in or on this membrane in the unulcerated regions studied. Only a few large bacilli were found lying on the surface of shallow ulcerations. They had not penetrated below the surface and were not considered to be typhoid bacilli.

On examination of the cellular foci, composed chiefly of lymphocytes and plasma cells, it soon became apparent, however, that young plasma cells occasionally contain within their rather purplish cytoplasm circumscribed areas filled with a lighter staining material

embedded in which are numerous, small, rod-shaped and spherical structures that are obviously bacteria.

Owing to the relatively deeply staining cytoplasm of the plasma cells and the small size of the intracellular bacteria the cellular inclusions are quite inconspicuous, but when found they are very definite. Search was then made through many sections of the intestinal lesions, and although the number of demonstrably infected plasma cells is small in any one section, typical examples were found in each. Similar plasma cells with intracytoplasmic bacillary and coccoid inclusions were likewise found in mesenteric lymph nodes draining the intestinal lesions and showing the usual typhoidal lesions. In no other lesions, including those of the spleen, liver and bone marrow, were intracellular bacillary inclusions demonstrated, although a careful examination was made of several sections from each of these tissues.

The intracytoplasmic bacillary inclusions were found only in plasma cells, and in very rare instances in cells with more abundant and more pink staining cytoplasm which might be interpreted as being altered plasma cells.

The interpretation that the cells containing the inclusions are plasma cells is based on the fact that they possess an eccentric round or oval nucleus with chromatic material generally arranged about the periphery, and a relatively abundant basophilic cytoplasm sometimes exhibiting a crescentric, paranuclear, slightly acidophilic area. These cells are commonly and most numerously situated within the persisting lymphoid aggregates of the intestinal follicles, and sometimes exhibit multiple or lobulated nuclei. All of these are commonly known characteristics of plasma cells; but the cells under consideration are rarely classical mature plasma cells. Ordinarily they seem larger with more abundant cytoplasm and are apparently immature. Mitotic figures in such cells were occasionally observed. Cells having the characteristics as described are commonly accepted by pathologists as plasma cells in their descriptions of similar lesions of typhoid fever.[3] They are never seen to contain phagocytosed material other than the bacillary aggregates which I have described. It is important that the identity of these cells be recognized because they are the only cell in the typhoidal lesion that contains the inclusions and this, therefore, appears to be a cellular specific phenomenon.

After the discovery of the small bacillary aggregates within plasma cells search was made for bacillary groups such as have been described by others in lesions of typhoid fever — namely extracellular aggregates that appear to have no obvious relation to the lesion. Such groups, though quite rare and exceedingly small, consisting of about a dozen or less bacilli, were found after careful search but only in association with and practically always within a necrotic typhoidal macrophage. On rare occasions a normal appearing macrophage contained a few bacilli in relationship with the partially digested remains of a phagocytosed lymphocyte. The bacillary forms within the dead macrophage differ considerably from those within the plasma cells. They are composed of large, deeply staining rods which are vegetative, as judged from their fresh appearance and the linking of one rod to another. Bacillary forms of this kind were found only within or near the inflamed follicles of the Peyer's patches.

The question naturally arises whether or not any or all of the bacillary groups, including the large and small forms, are typhoid bacilli. Shortly before death in the case under consideration a blood culture yielded only *E. typhi*, 60 colonies per cubic centimeter. Clinically there was no evidence of a complicating infection. The case was such an early one and the tissues were fixed so shortly after death that there is no evidence microscopically of invasion of the intestinal lesions by any secondary bacteria. The bacillary inclusions within plasma cells are Gram-negative and resemble individually and collectively the small intracellular bacillary forms encountered in the entodermal epithelium of the chick embryonic membranes inoculated with *E. typhi* and from which pure cultures of this bacillus were recovered. Furthermore, the plasma cell is not known to be an active phagocyte, nor does it seem to migrate extensively. Those that contained bacterial inclusions in the intestinal lesions were most commonly situated near the central portion of follicular remnants, often remote from the intestinal surface, and they were also present in mesenteric lymph nodes that have been found to yield pure cultures of *E. typhi*.

Such considerations lead me to the conclusion that these represent an especially small bacillary form of *E. typhi* modified by their intracellular environment. That they are reproducing within the cytoplasm of the host-cell is judged by the fact that the focal aggre-

gates consist of numerous individuals. It often appears that they are embedded in a capsular material, possibly produced by themselves. There are no degenerating forms and they are not surrounded by a digestive vacuole, nor are they irregularly distributed in the cell, but occur as focal circumscribed inclusions. Furthermore, there are no detectable extracellular bacteria from which the incorporated forms might have been derived by ingestion.

It is of interest that in no instance has there appeared a large bacillary form or group derivable from a plasma cell. There is no evidence of an ante or postmortem transformation of the small forms into the large within the plasma cell. On the other hand only the large bacillary forms are found within the typhoid macrophage. Whether these represent postmortem growth or not cannot definitely be determined, but one has reason to judge that they do represent an ante mortem growth.

In the first place they are practically always associated with and incorporated by macrophages that exhibit evidences of necrosis. They are quite rare, more so than the plasma cell forms. There is no extracellular growth and in view of the abundant microorganisms in the blood stream it would seem, had postmortem growth occurred, that extracellular foci would be in evidence. Failure to find bacterial foci in the spleen and bone marrow is also opposed to an interpretation of postmortem growth.

Although the foregoing description is based largely on observations made on the tissues of the case reported, plasma cells containing characteristic intracytoplasmic groups of Gram-negative bacilli, judged to be *E. typhi*, have been found in Peyer's patches of 4 additional cases of typhoid fever. The late stages of the disease with complete ulceration, and tissues from cases examined several hours after death, are not suitable for such a study. No such inclusions have been found in tissues from other conditions, including lesions of bacillary dysentery, satisfactorily prepared for their demonstration.

## Discussion

Observations of the phenomena of typhoid fever up to the present time have neither indicated the portal of entry of the typhoid bacillus into the tissues of the human host nor the sites of multiplication of this microorganism after invasion has taken place. Evidence

indicates that *E. typhi* does not multiply within the lumen of the gastro-intestinal tract, or within the blood stream.[5,2]

Although the observations I have recorded do not indicate the portal of entry, they may be interpreted to point out a site and mechanism of growth of the causative agent after invasion has taken place — namely within the cytoplasm of living young plasma cells, and within the cytoplasm of living or dead macrophages in the lymphoid tissue of the intestine and mesenteric lymph nodes.

The Gram-negative, small bacillary microorganisms found in groups within apparently normal and uninjured young plasma cells are interpreted to be intracellular forms of *E. typhi*. Similar structures have not been found in other cells, and it is tentatively concluded that the human host offers at least one living intracellular medium to which this bacterium may gain access, be thereby protected from unfavorable extracellular influences, and multiply. A few cells, presumably plasma cells, containing relatively large aggregations of bacilli appear to be necrotic, and one may assume that the enclosed bacilli may be liberated by rupture or disintegration of plasma cells thereby to gain access to the lymph and blood stream, or to become phagocytosed by other cells of the same kind in which the processes of multiplication may be continued in series.

On the other hand, the liberated bacilli could be phagocytosed by the macrophages, for Gram-negative bacilli, judged to be typhoid bacilli, have likewise been found in these cells. But the macrophage does not seem to be naturally a suitable medium for growth of the bacillus. In those instances where evidences of growth in macrophages are apparent the bacilli are associated with the digesting remains of a phagocytosed lymphocyte or are incorporated within the cytoplasmic remains of a dead macrophage.

Infection of embryonic chick membranes indicated that *E. typhi* multiplied under two conditions: (1) in association with necrotic tissue, and (2) within the cytoplasm of living entodermal cells. Within the intestinal lesions of typhoid fever similar conditions seem to exist. But here the living receptive and nourishing cell is the young plasma cell, and the necrotic material is the intracellular remains of a phagocytosed cell or the autolysing remains of the phagocyte itself.

In the infected embryonic tissue the bacilli multiplying within living entodermal cytoplasm are quite small and are embedded in an

incorporating material, while those growing in necrotic tissue are large and free. A similar distinction is found in the human lesions, namely the bacilli within plasma cells are small and lie within an incorporating material, while those within macrophages are large bacilli and free.

There are only a few bacilli, however, within a single macrophage, no more than might be considered to be subsisting on the dead material with which they are associated. It is rare to find a macrophage that contains bacilli, and one may reasonably judge that the microorganism is protected and nourished by the disintegrating cells for only a brief period. It seems probable that, following a short series of divisions, the bacilli become, through dissolution of the incorporating macrophage, exposed to the extracellular environment, whence they may reenter either young accessible plasma cells to repeat a generative cycle, or a macrophage (where they may be destroyed), or may regenerate, if the cell dies or is digesting a phagocytosed lymphocyte. On the other hand, if humoral bacteriolysins have developed the exposed bacilli may be rapidly dissolved, liberating thereby their endotoxins.

The "positively chemotactic" and susceptible young plasma cell within lymphoid tissue generally could come in contact with *E. typhi* and become infected, either from direct invasion of the intestinal wall or by indirect invasion through the blood stream. Consequently without knowledge of the earliest stages of infection it is not possible to be certain of the portal of entry. It would seem reasonable to conclude, however, that injury of lymphoid tissue resulting in proliferation of young plasma cells would predispose those tissues to the most extensive infection; and experience shows such injury occurs most prominently in the lymphoid tissue of the lower ileum and the mesenteric lymph nodes draining this area.

No bacillary groups were found in the early cases in any tissue examined other than the lymphoid tissue of the ileum and mesentery. It seems quite probable that extracellular bacilli would form small colonies in the dead tissues if they were permitted to incubate long enough, and observations of others indicate that they do so.

If it is assumed that the young plasma cells are the susceptible hosts for growth of *E. typhi* during the incubation period of typhoid fever, and from them the bacilli become disseminated, primary or metastatic infections might occur in various parts of the

body, notably in the tonsils, pharyngeal lymphoid nodules, intestine, lymph nodes, spleen and bone marrow, where plasma cells are normally present.

In the early case under examination no bacilli were found in the spleen, liver and bone marrow. The focal necroses present in these structures rather indicate the effects of endotoxins from phagocytosed and lysed bacilli from the circulating blood. Plasma cells are quite rare in the liver and *E. typhi* is almost never recognized in this organ although focal necroses are numerous. These cells, however, are more abundant in the spleen and bone marrow and, although no intracellular bacilli were found, it seems possible that cycles of generation could occur in these tissues within plasma cells.

Persistence of typhoid fever after the disappearance of the specific bacilli from the blood stream might be explained by a continuance of growth within plasma cells locally, although antibodies prevent their further dissemination.

The observation that the human plasma cell can be a susceptible cellular host for *E. typhi* may have a bearing on immunity to typhoid fever. It is generally conceded that recovery from typhoid fever is associated with a prolonged immunity to subsequent infection by *E. typhi*, while vaccination at best usually leads to less substantial and briefer immunity.

If infection is associated with intracellular multiplication of *E. typhi* it seems possible that antigens differing perhaps from those derivable from artificially cultivated bacilli may be liberated during the course of infection and that the susceptible plasma cell itself may become immune.

It is to be hoped that the observations I have recorded and the interpretation of them proposed may be subjected to more critical examination by others from investigation of the disease in man or in chimpanzees, in order that a better understanding of the pathogenesis of typhoid fever may be acquired and that the significance of relations between the cells of susceptible animals and parasitic agents may be demonstrated or excluded in this and other infectious processes.[6]

## Summary and Conclusions

1. In early cases of typhoid fever small Gram-negative intracellular bacilli, judged to be *E. typhi*, have been found at autopsy

in the cytoplasm of young plasma cells, otherwise apparently unaltered, located in the lymphoid follicles of iliac and mesenteric lesions.

2. Larger Gram-negative bacilli have been found in macrophages of the intestinal lesions in association with the remains of phagocytosed lymphocytes, or the necrotic remnants of macrophages themselves.

3. It is concluded that *E. typhi* is capable of growing in both these situations and under conditions indicated.

4. The interpretation is proposed that the young plasma cell is an essential cellular host for *E. typhi* in the typical human disease and serves as a nourishing and protecting medium, not only during the period of incubation but throughout the active course of the disease.

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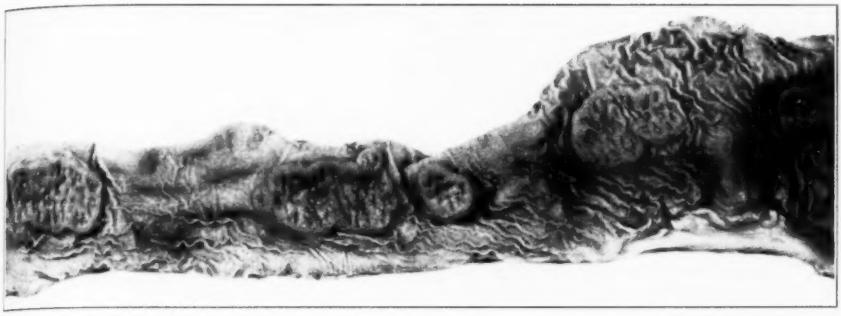
## DESCRIPTION OF PLATES

### PLATE 24

FIG. 1. Peyer's patches in ileum.

FIG. 2. Peyer's patch and solitary follicles just above ileocecal valve. Note slight ulceration.

FIG. 3. Peyer's patch showing partial covering with epithelial sheet. × 16.



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Goodpasture Pathogenesis of Typhoid Fever

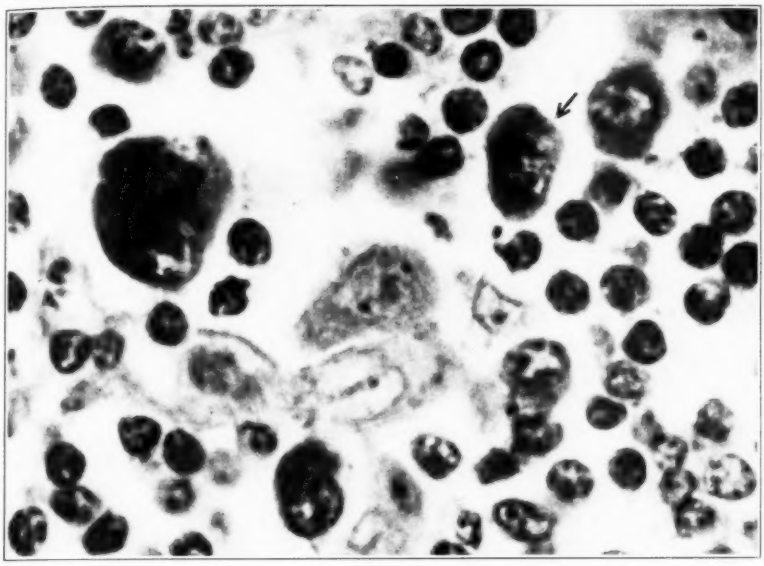
PLATE 25

FIG. 4. Photomicrograph showing young plasma cells near center of a persisting follicle in a Peyer's patch. Arrow points to ill-defined group of intracellular bacilli. × 1400.

FIG. 5. Arrow points to young plasma cell from a Peyer's patch containing a group of small bacilli. × 2500.

FIG. 6. Plasma cell with pyknotic nucleus. Intracellular group of small bacilli. × 2500.

FIG. 7. Necrotic macrophage containing group of large bacilli. × 2500.



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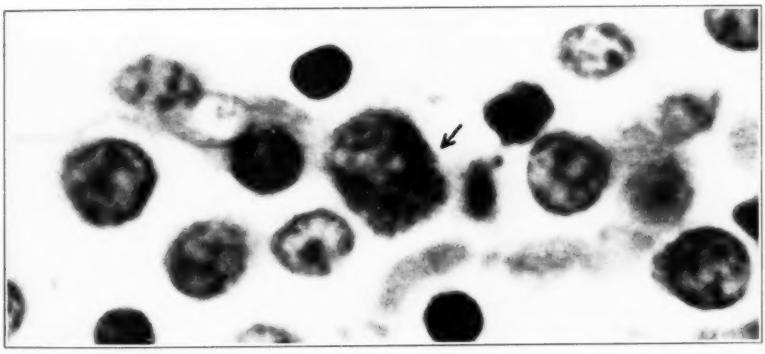

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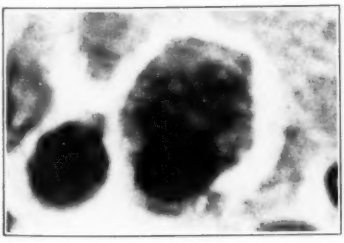

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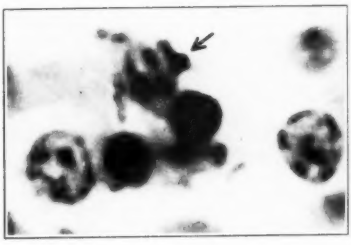

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Goodpasture

Pathogenesis of Typhoid Fever

PLATE 26

Cells from case reported. Figure 13 from a mesenteric node. Others from Peyer's patches.

FIG. 8. Plasma cell containing small bacilli. Large and small lymphocyte and macrophage.

FIG. 9. Group of young plasma cells. One near center contains a group of small bacilli.

FIG. 10. Plasma cell containing bacilli. Note encapsulating material.

FIG. 11. Necrotic plasma cell with encapsulated group of small bacilli.

FIG. 12. Plasma cell containing large group of bacilli.

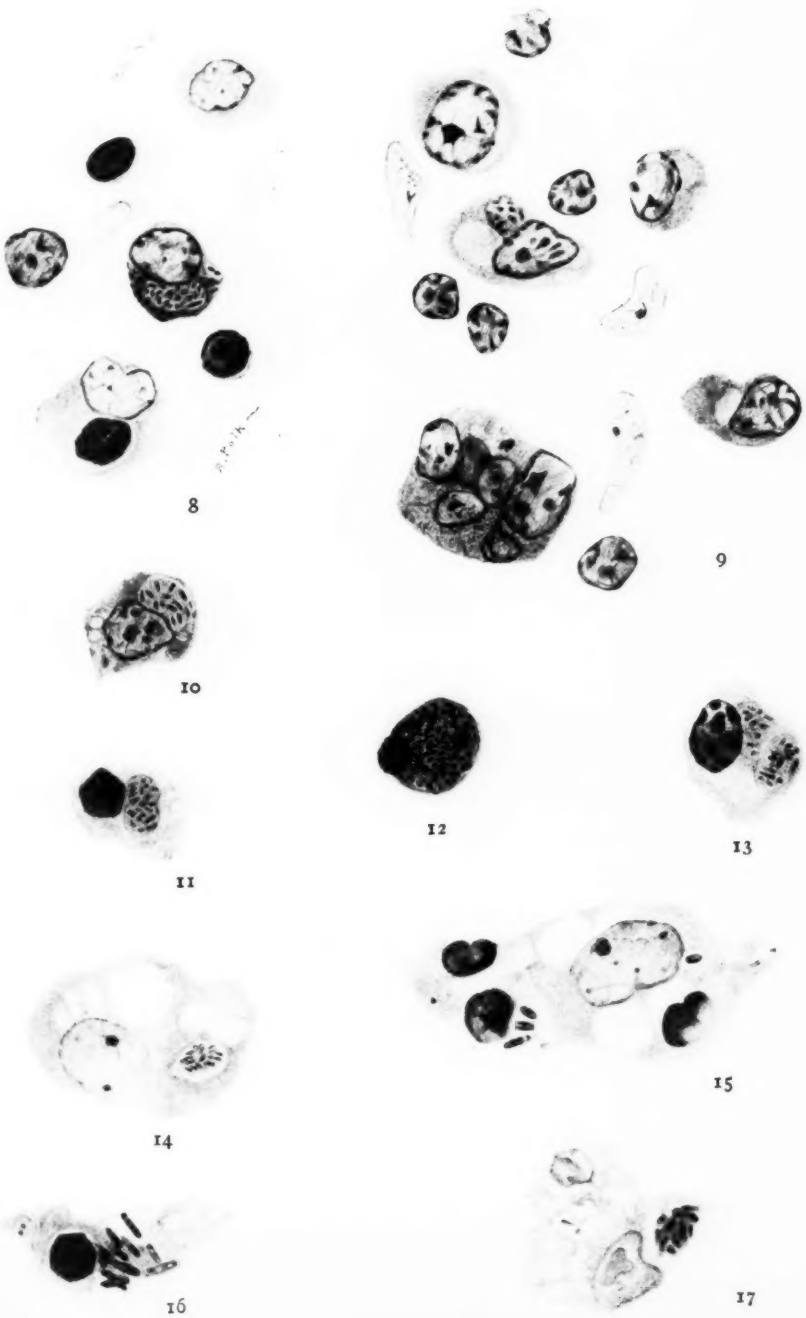
FIG. 13. Plasma cell containing two groups of small bacilli.

FIG. 14. Large cell, possibly transformed plasma cell containing group of small bacilli.

FIG. 15. Macrophage containing dead lymphocytes associated with one of which is a small group of large bacilli.

FIG. 16. Dead macrophage containing large bacilli.

FIG. 17. Dead macrophage containing large bacilli.



# THE HISTOPATHOLOGY OF NATURAL AND EXPERIMENTAL CANINE DISTEMPER*

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Canine distemper is a highly infectious disease, the most constant manifestation of which is a characteristic temperature curve. According to Lockhart,[1] who has observed several thousand cases in young dogs, there is an initial rapid rise in temperature which occurs approximately 7 to 8 days after exposure to infection. After an interval of about 96 hours the temperature recedes to practically normal, only to become elevated again on the 11th to 12th day following exposure. There is usually an associated coryza of a varying degree, and many cases show striking suppurative catarrhal symptoms such as purulent discharges from the eyes and nose. Bronchitis is frequent and often in fatal cases there is bronchopneumonia. Vesicopustules often occur on the abdomen. Diarrhea is at times a prominent symptom and results in severe emaciation. In some outbreaks of the disease as many as 50 per cent of the cases develop nervous manifestations which may result in fatalities as high as 75 per cent. The nervous symptoms usually develop after the 2nd week of the disease and consist of sudden attacks of semiconsciousness with vigorous chewing movements and salivation. At first these attacks last only a few seconds, after which the dog regains consciousness. Usually when such fits set in they become more frequent and severe, finally progressing to the stage of violent epileptiform convulsions. Some cases exhibit muscular spasms which may progress to the stage of generalized convulsions. A small proportion of cases of distemper are so mild as to escape recognition, but as a rule some of the symptoms are pronounced, particularly those referable to a particular system. For this reason many veterinarians have divided the clinical types into pulmonary, intestinal, tegumentary or nervous, depending on the system producing the outstanding symptoms.

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## Susceptible Animals

Laidlaw[2] is of the opinion that all dogs, with the exception of young suckling puppies and those immunized by a previous attack of the disease, are subject to distemper. He also regards ferrets, stoats and weasels as being highly susceptible. Because of the extreme susceptibility of the ferret the English variety of this animal was extensively used by Dunkin and Laidlaw[3] in their experimental study of distemper. According to Darling[4] the brown or fitch ferret is equally susceptible, and responds to the infection in a manner identical with that of its close relative. Laosson[5] reported the successful transmission of the disease to two foxes. Fox[6] reported the occurrence of the disease in wild dogs, wolves, lynxes and racoons, in captivity.

## The Etiological Agent

Carré[7] in 1905 reported that distemper of dogs is caused by a filterable virus. Ferry[8] and M'Gowan[9] in 1911 independently described a small Gram-negative bacillus which they isolated from the upper respiratory tract and which was interpreted by them as the probable etiological agent of the disease. This organism was later named *B. bronchisepticus* by Ferry.[8] Torrey and Rahe[10] (1913) studied this organism thoroughly and claimed that they reproduced typical distemper in susceptible dogs by injecting them with pure cultures of the organism. Dunkin and Laidlaw [3,11] (1926), however, regard the bacillus as a secondary invader which is often responsible for the pulmonary complications of the disease. These investigators, working with a stock of ferrets and dogs bred and reared under the strictest possible isolation, substantiated the earlier work of Carré[7] in proving conclusively that the etiological agent of the disease belongs to the class of filter-passing viruses.

## Distribution of the Virus

The virus of canine distemper apparently is generally distributed throughout the tissues of the infected animal. Carré[7] (1905) demonstrated its presence in the blood, pericardial fluid and nasal secretions of infected dogs in sufficient quantities to reproduce the disease in susceptible animals. Puntoni[12] (1923) found the brain to be infective, and Dunkin and Laidlaw[3] (1926) succeeded in inducing dis-

temper in ferrets with as little as one-ten-thousandth of a gram of splenic tissue. The wide distribution of cell inclusions reported in the histological studies of some investigators, granting that these inclusions bear a close relation to the virus, also indicates that there is a more or less general distribution of the virus in this disease.

## Histology and Cytology

In investigations of the histopathology of canine distemper the brain has been studied more than other organs, perhaps because (1) the symptoms in the nervous type of the disease are so striking, and (2) because of the desire for a clear differentiation between the histopathology of this disease and that of rabies.

Lentz in 1907 found in the nervous type of distemper of young dogs a degeneration of nerve cells of the brain, especially those in Ammon's horn, and small, cytoplasmic acidophilic inclusions within the neurons. These inclusions, some of which seemed to lie outside cells, appeared quite similar to Negri bodies, but they contained no internal structure. In 1909 [13] he published a detailed account of his findings, which had been confirmed by Standfuss in 1908.[14] Golgi and Sinigaglia,[15] and Babes and Starcovici [16] in 1912 described small inclusions, with a vacuolated structure resembling Negri bodies, in the Purkinje cells, in the nerve cells of the spinal cord, in the ependymal cells of the ventricles, and in the epithelium of the respiratory tract and conjunctiva. Sanfelice [17] in 1915 found similar cytoplasmic inclusions in 7 cases in nerve cells, in the nasal mucosa, conjunctiva, intestine, spleen, pancreas, skin, lymph nodes, bone marrow, ovary and lung, but did not find them in the liver, kidneys or salivary glands. Kantorowicz and Lewy [18] in 1923 described intracellular structures occurring in the brains of dogs that had the nervous form of distemper. In their opinion these resembled the "parasite" *Encephalitozoön cuniculi* which commonly occurs in the brains of rabbits. Roman and Lapp[19] in 1925 studied the central nervous systems of 27 dogs suffering from canine distemper. Of this number, 19 showed changes in the central nervous system in the form of a disseminated non-suppurative process of the cord, brain and leptomeninges without associated cell inclusions. Pugh[20] in 1926, in a study of over 50 fatal cases in an epidemic of encephalitis in dogs, found in the lethargic cases, especially in the basal ganglia, an exten-

sive perivascular reaction associated with minute hemorrhages. Because of the similarity of these lesions to those of some cases of human encephalitis, he seriously considered a possible relation between the two diseases. It seems, however, that the disease described by Pugh is not distemper, as Laidlaw did not succeed in infecting ferrets with brain tissue obtained from some of the cases; and also it was determined that the disease occurred in some dogs that had recovered from distemper and were, therefore, immune to that infection.[2] Dunkin and Laidlaw[3,11] in 1926, following their extensive study of canine distemper, concluded that histological examination shows no characteristic lesions. They noted hyperplasia of the endothelial cells of the lymphatic system. In the spleen, abdominal lymph nodes and Peyer's patches some of these cells showed degenerative changes. Others contained phagocytosed red blood cells and leukocytes and, very occasionally, irregularly shaped, eosinophilic cytoplasmic inclusions. They also observed, but very rarely, eosinophilic inclusions in the cytoplasm of epithelial cells of the conjunctivae and pulmonary alveoli. Because bronchopneumonia, purulent conjunctivitis and pustules on the abdomen, all of which are commonly seen in the natural disease, did not occur in their experimental animals, these features were regarded as complications due to infection by secondary organisms. In cases of the nervous type of the disease they found evidences of a non-specific encephalitis but noted no inclusions in nerve cells.

Dunkin and Laidlaw[3] also studied distemper in ferrets and described lesions quite similar to those occurring in dogs with the experimental disease. In some of these animals they noted degeneration of nerve cells of the brain, usually without any associated inflammatory reaction. Also they described multiple, small epithelial abscesses which occurred constantly in the lips and occasionally on the abdomens of ferrets. Occasional cytoplasmic inclusions were found in epithelial cells of the walls of these abscesses. These investigators concluded that in the experimental disease of both dog and ferret inclusion bodies do not occur with the regularity of number, size and disposition which is to be expected if they are directly associated with the infecting agent. Marinesco, Draganesco and Stroesco[21] in 1933, in a study of the central nervous systems of 20 dogs with distemper, noted in the brain rather diffuse degenerative

and inflammatory lesions with perivascular accumulations of lymphocytes and clasmatocytes. The inclusions which they described occurred exclusively in the nuclei of nerve and glial cells. Nicolau[22] in 1935 described 4 fatal cases of distemper in which cytoplasmic inclusions, similar to those previously described by Lentz, were widely distributed, and occurred in cells derived from all three types of embryonic tissue. He found nuclear inclusions, however, only in glial and nerve cells.

While canine distemper and its inciting agent are now well recognized as clinical and virus entities, our knowledge of its histopathology, as indicated by the above review, is still unsatisfactory. The object of this paper is to report the results of studies of the natural and experimental disease which we have undertaken in the hope of obtaining a more thorough understanding of its cytology and histology.

## The Natural Disease

### *Materials*

This study is based on the findings in 13 fatal cases of distemper in young dogs. In order to avoid repetition in description, these dogs have been divided into 3 groups, dependent on the important clinical features which they manifested. Group I consists of 5 dogs that suffered from the so-called catarrhal form of distemper, without developing symptoms referable to the central nervous system. Group II consists of 7 dogs that suffered from the typical nervous form of the disease characterized, in each case, by severe generalized convulsions. Of this number, 5 were supplied to us by experienced veterinarians who regarded them as typical cases of the nervous type of distemper. The other animal, comprising Group III of the series, was a $5\frac{1}{2}$ weeks old Boston bull puppy which died after an illness of about 30 hours. At the onset of illness this puppy was extremely nervous and refused all food and water. It rapidly weakened, became lethargic, and died in coma without evidence of paralysis or convulsions. This puppy had one litter-mate and it, too, died following a similar illness of short duration. The mother of these puppies developed a non-fatal illness, which was probably distemper, about 10 days before the puppies became affected.

*Gross Pathology*

All of the dogs included in Groups I and II were notably emaciated and dehydrated. In each of these cases the thymus was quite edematous and the amount of pericardial fluid was slightly increased. The mesenteric lymph glands were soft and swollen and the spleen was pale and slightly enlarged. In most cases there were small punctate hemorrhages in the mucosa of the small and large intestine, in the pericardium and in the pleura. In 2 of the cases there was a rather extensive purulent bronchopneumonia associated with fatty degeneration of the liver. In no case did examination of the brain reveal anything of significance.

The dog comprising Group III was in a good state of nutrition. Autopsy of this animal also revealed an edematous thymus, an increase in pericardial fluid, an enlarged pale spleen and soft swollen mesenteric lymph nodes. In addition the lungs appeared hemorrhagic. The liver was much enlarged and extremely friable. There was a large amount of clear fluid in the peritoneal cavity, and innumerable minute hemorrhages were seen in cut surfaces of the brain.

*Histological and Cytological Studies*

Blocks of tissue from the various organs, including in most instances tissue from representative areas of the brain, and in many instances spinal cord and dorsal root ganglia, were fixed in Zenker's fluid containing 5 per cent acetic acid. Microscopic sections of this material were stained routinely with hematoxylin and eosin. In a few instances, to be designated, special stains were employed. The significant histological and cytological findings are recorded below.

*Lungs:* The lungs of all the animals in Groups I and II presented an interstitial pneumonia that was most distinct about the bronchioles. In these areas the peribronchial tissues and the walls of the adjacent alveoli were infiltrated with mononuclear cells. The mucous membranes of the larger and medium sized bronchi were slightly infiltrated with mononuclear cells and, in many instances, the mucous membranes of the smaller bronchi were completely denuded of epithelium. Desquamated alveolar epithelial cells were unusually abundant in some areas. Even in the 2 cases that presented a definite purulent bronchopneumonia there were numerous areas in which the above mentioned changes were not obscured by the complicating

infectious process. In most cases small areas of hemorrhage were noted. In all cases cytoplasmic inclusions were found in both bronchial and alveolar epithelial cells. In some bronchi these were especially abundant. They were situated in definite vacuoles and appeared as well defined, usually round to oval, sometimes elongated, vacuolated acidophilic bodies most often lying between the nucleus and the outer margin of the cell, but sometimes at the opposite poles of the cell. Not uncommonly as many as three of these bodies occurred in a single cell. Their vacuolated appearance and well defined contours caused these inclusions to bear a striking resemblance to the Negri bodies of rabies. We regard them as being identical with the inclusion bodies previously described by Golgi and Sinigaglia[15] and others. Nuclear inclusions were also present, but in less abundance, in epithelial cells of the bronchi and of the mucous glands. A nucleus which contained this type of inclusion was swollen and appeared as a ring of chromatin enclosing a vacuole in which was situated the acidophilic inclusion body. These inclusions were quite variable in size, but in contrast to the cytoplasmic inclusions they were quite irregular in outline and homogeneous. Occasionally both cytoplasmic and nuclear inclusions occurred within the same cell.

Sections of the lungs of the puppy in Group III also revealed similar cytoplasmic and intranuclear inclusions in bronchial and alveolar epithelium. The endothelial cells of the alveolar capillaries were considerably swollen and occasionally, within cells of this type, there occurred single, homogeneous, acidophilic nuclear inclusions which were fairly regular in outline and of a size sufficient to fill half the nucleus.

Because of the constancy with which both nuclear and cytoplasmic inclusions appeared in the bronchial epithelium of dogs that died of distemper, their occurrence in these cells was thought to be characteristic of the disease. In order to obtain further evidence that this is the case we examined microscopic sections of lungs removed from 18 normal adult dogs and from several other dogs suspected of having rabies. These inclusions were not found in any of the control animals.

*Liver:* The livers of all dogs in Groups I and II showed increased cloudy swelling, and three showed a moderate degree of fatty degeneration. In some cases there were small areas of focal necrosis in

which mononuclear cells occurred. Both cytoplasmic and nuclear acidophilic inclusions, similar to those found in bronchial epithelium, were present in epithelial cells of the bile ducts in all cases. These were abundant in 2 cases, but their presence in the remainder of this group was detected only after prolonged search. No inclusions were detected in liver cells or Kupffer cells. Focal necrosis was somewhat more pronounced in the liver of the puppy of Group III. A striking feature in this liver was the presence of acidophilic nuclear inclusions within the liver cells and Kupffer cells. Almost every liver cell, and the great majority of the Kupffer cells, contained a single, large, homogeneous, fairly sharply outlined, round to oval inclusion which was usually centrally located. These inclusions lay in vacuoles that pushed the basophilic nucleoli to one side, frequently against the condensed peripheral ring of chromatin that appeared to form the wall of the swollen nucleus.

*Spleen:* The spleens from all cases in this series showed enlarged malpighigian follicles composed almost entirely of a central mass of pale staining, proliferating mononuclear cells. The lymphoid tissue was notably reduced in amount and confined to the periphery of the follicles. Many of the mononuclear cells were necrotic; others contained the remains of phagocytosed red and white cells. Usually within each follicle there occurred from one to a half-dozen or more mononuclear cells with swollen, irregularly shaped nuclei that contained acidophilic inclusions similar in every way to those in the Kupffer cells of the liver of the puppy of Group III. Occasionally these inclusions were detected in endothelial cells lining the sinusoids. Small hemorrhages were occasionally seen in both follicles and splenic pulp.

*Mesenteric Lymph Nodes:* In all cases of this series the reticulo-endothelial cells of the mesenteric lymph nodes, as in the spleens, showed considerable activity and a diminution of lymphocytes. Nuclear inclusions in the reticulo-endothelial cells, identical with those occurring in the spleen, were constantly detected, usually in abundance. Also, in the cytoplasm of these cells small, red staining, sometimes vacuolated bodies have been frequently detected, but as yet we have been unable to differentiate them satisfactorily from fragmented red cells.

*Intestine and Stomach:* Minute hemorrhages were frequently observed in the mucous membranes of the stomach and intestine but

definite ulceration did not occur. From 1 dog a section of colon, which at autopsy contained a large amount of bloody mucus, showed an extensive infiltration of the submucosa by mononuclear cells, many of which contained nuclear inclusions. Also, nuclear inclusions were detected in glandular epithelial cells of the colon and in the chief cells of the gastric mucosa of this dog and 1 other.

*Central Nervous System:* Sections of the spinal cord from dogs in Groups I and II and of the dorsal root ganglia of those in Group II revealed nothing of significance. In 1 case the meninges covering the cerebellum near the brain stem were infiltrated with mononuclear cells, some of which contained nuclear inclusions similar to those occurring in endothelial cells of the spleen and the lymph nodes. In the underlying cerebellar tissue, as far as the line of Purkinje cells, there was definite proliferation of both neuroglial and microglial cells, and in each of these types of cells both nuclear and cytoplasmic inclusions were detected, usually in the same cells. The nuclear inclusions appeared as single, acidophilic homogeneous bodies often quite irregular in outline and easily demonstrable in hematoxylin and eosin preparations. The cytoplasmic inclusions, of which as many as three were sometimes seen in a single cell, appeared as small, vacuolated acidophilic bodies. They were shown to better advantage in preparations stained with Goodpasture's carbol aniline fuchsin and with Wright's stains. In preparations stained by the latter method vacuoles and definite inner bodies, such as those occurring in Negri bodies, could be detected. Inclusions in nerve cells were not noted in this case.

In 2 other cases of Group II, however, both types of inclusions were detected in nerve cells, as well as in neuroglia and microglia. The nerve cells showed evidences of degeneration with neuronophagia. In 1 dog the lesion was detected in Ammon's horn, and in the other in the basis pedunculae on one side. In these 2 cases the meninges and choroid plexuses appeared normal. Significant findings were not detected in sections of the central nervous systems of the other 2 dogs in Group II.* Sections from all parts of the brain and cord of the dog of Group III showed numerous minute hemorrhages with swelling and some proliferation of the capillary endothelial

* Recently we have found cytoplasmic and nuclear inclusions in three additional brains of dogs with the nervous form of distemper. This strengthens our belief that focal lesions of this type are characteristic of this form of the disease.

cells. Some of these swollen endothelial cells contained nuclear inclusions identical with those seen elsewhere in endothelial cells. No inclusions were found in nerve cells in this case.

*Other Organs:* The *kidneys* of all the animals in this series showed considerable cloudy swelling. In the case of the puppy of Group III from one to four endothelial cells of every glomerular tuft were seen to contain nuclear inclusions. An occasional medullary cell of the *adrenals* in this case also contained a nuclear inclusion.

## THE EXPERIMENTAL DISEASE

### *Materials and Methods*

Because distemper is so prevalent, most full-grown and many half-grown dogs have had the disease and are, therefore, immune. Realizing this we used in our experiments very young puppies from 3 to 10 weeks old and obtained as far as possible from the surrounding country districts. Only healthy puppies in good physical condition were used.* Following this plan practically all animals used proved to be susceptible to distemper. In order to avoid, as far as possible, the complication of spontaneous infection in the experimental animals they were inoculated immediately after being brought into the laboratory, usually with large doses of virus. Puppies that received a large amount of virus developed a fatal disease which ran a very rapid course. In cases where small amounts of virus were injected, because of the longer course of the disease, the animals were isolated and cared for by a special attendant. Cages and vessels used for food and water were sterilized in an autoclave.

The strain of virus largely used in this study was obtained from the spleen of the Boston bull puppy of Group III. It is to be recalled that the disease in this animal ran a very acute course, and at autopsy nuclear inclusions were found in great abundance in liver cells, Kupffer cells and in endothelial cells elsewhere. In all, a total of 53 dogs have been injected with this strain. Approximately half of this number were injected intraperitoneally with suspensions of

* During the extremely hot weather prevalent in this community during the last few weeks it became increasingly difficult to obtain puppies that were not already suffering from distemper. For that reason this investigation has been suspended until cooler weather when, it is believed, an adequate supply of susceptible animals will again be available.

liver tissue containing the virus in order to propagate the strain and to obtain material for histological examination. Using this method this strain of virus has been carried through 16 generations. In addition the same virus was injected by a variety of routes.

One other strain was used only to establish its identity with the strain already mentioned. The second strain was obtained from the lung of a dog that had the typical nervous form of distemper. In addition to numerous cytoplasmic and nuclear inclusions in the bronchial epithelium of this dog, both cytoplasmic and nuclear inclusions were present in the brain in nerve cells, microglia and neuroglia. Also, there were nuclear inclusions in reticulo-endothelial cells of the spleen and mesenteric lymph nodes, but no inclusions were detected in liver cells. One cc. of a 15 per cent emulsion of lung tissue of this animal was injected intravenously into a small puppy. Eight days later, when the puppy died, autopsy revealed nuclear inclusions in the liver cells and Kupffer cells, and the other features presented by puppies that had received intravenous injections of the first strain of virus. The second strain was injected in large amounts, in series, into 2 other puppies with the same results. This strain was then discarded as it seemed that the two strains were identical.

### *Experiments and Descriptions of Gross and Microscopic Pathology*

#### *Intraperitoneal Injections*

During the course of these experiments 17 young puppies were inoculated intraperitoneally with saline suspensions of fresh liver containing nuclear inclusions in liver cells and Kupffer cells. The amounts injected varied from 0.5 to 4 cc. of a 15 per cent suspension. Puppies that received the larger amounts of virus usually appeared normal for 24 hours or so after inoculation. They then became restless and refused all food and water. After remaining in this condition for 24 to 48 hours they passed rapidly into coma. Death usually occurred during the 3rd day following inoculation. At autopsy it was the rule to find a cloudy, bacteria-free peritoneal fluid, sometimes in considerable amount. The peritoneal surfaces were, for the most part, covered by a fibrinous exudate. The liver was much enlarged and very friable and its lobes were plastered together with exudate. The spleen was also enlarged and quite pale. The mesenteric lymph glands were extremely swollen and, at times, hemor-

rhagic. Small hemorrhages in the mucosa of the stomach and intestines, and in the lungs, were quite common.

With the exception of the evidences of peritonitis, the histological and cytological findings in these animals were almost identical with those found in the puppy that died of the natural disease, and served as the source of virus used in these experiments. In sections of intestine, diaphragm, and other structures that contained peritoneum, many serosal cells were seen containing nuclear inclusions similar to those in endothelial cells of the natural case. Serosal cells, often containing nuclear inclusions, were seen wandering into the underlying tissues, especially in such loose tissue as the subserosa of the intestine and the diaphragm. The cellular elements of the exudate present on the peritoneal surface consisted of mononuclear cells and polymorphonuclear leukocytes in about equal proportions. The liver cells were swollen and, in some cases, practically all contained nuclear inclusions. The Kupffer cells were also swollen, showed evidences of proliferation, and the majority contained nuclear inclusions. The portal areas were infiltrated with mononuclear cells, some of which also contained inclusions. Inclusions were not present in bile duct epithelium. In some instances the liver cells contained an abundance of fat globules but necrosis was uncommon.

The microscopic pathology of the spleens, mesenteric lymph nodes, lungs and kidneys of these puppies was identical with that described in the puppy from which the strain of virus was obtained, and nuclear inclusions were detected, usually in abundance, in endothelial cells of these organs. Occasional nuclear inclusions also occurred in medullary cells of the adrenals. The meninges in all cases appeared normal, but occasionally a few microscopic hemorrhages were seen in the brain and spinal cord. Usually, in such cases, the vascular endothelium of the capillaries of the brain appeared swollen and occasionally contained nuclear inclusions. Puppies that received smaller intraperitoneal injections of virus usually lived 8 to 15 days, and in these animals necrosis of liver cells, commonly of a midzonal distribution, was pronounced. The necrotic areas were frequently heavily infiltrated with mononuclear cells. Regeneration of liver cells was often evident. Nuclear inclusions were usually detected in liver cells and Kupffer cells. It is our impression that the inclusions persist longer in endothelial cells than in liver cells. They were found in endothelial cells of the spleen and lymph nodes in all

cases, although in a few they were not found in liver cells or Kupffer cells.

One puppy was given an intraperitoneal injection of 3 cc. of urine obtained at autopsy of a puppy that died 3 days after virus-containing liver was injected into the subcutaneous tissue of the abdomen. This puppy died 18 days later. Microscopic sections revealed nuclear inclusions in endothelial cells of the spleen and mesenteric lymph nodes; but unlike cases of the natural disease, inclusions were not detected in liver cells, Kupffer cells or bronchial epithelium.

Another puppy was given an intraperitoneal injection of 5 cc. of blood obtained from a moribund puppy that had, 15 days previously, been injected with a Berkefeld filtrate of liver tissue that contained numerous inclusions. For approximately 2 weeks before it died, 72 days after inoculation, this puppy was extremely spastic throughout, exhibited a lateral nystagmus and a tendency to turn the head to one side. Autopsy revealed the features already described in puppies that received smaller intraperitoneal injections of virus. In addition a lesion responsible for the neurological signs was found in the brain. In the lower medulla this lesion was largely in the dorsolateral portion of the medulla in the region occupied by the spinocerebellar paths. On one side it extended as far ventrally as the inferior olive and as far dorsally as the restiform body. On the opposite side the lesion was smaller and neither the olive nor the restiform body was appreciably involved. In the pontine region the brachia pontis were extensively involved and the pons itself to some extent. In the mesencephalon the lateral and medial portions of each basis pedunculae as high as the internal capsule showed extreme destruction. There was no obvious involvement of the corticospinal paths. In the involved area both neuroglia and microglia were greatly increased in numbers, and the latter were filled with lipoid globules. Nerve cells, where present, were in various stages of degeneration. Nuclear and cytoplasmic inclusions identical with those found in the brains in some of the cases of natural distemper of the nervous type were found in neuroglial, microglial and nerve cells. The overlying meninges and the tela choroidea were heavily infiltrated with mononuclear cells, many of which contained nuclear inclusions. Thrombi were not found in any of the vessels, but some vessels were almost occluded by swollen, proliferating endothelial cells. Many

large mononuclear cells were seen infiltrating the tissues surrounding some blood vessels.

*Intravenous Injections*

Three puppies were injected intravenously, 2 with 1 cc. of a 15 per cent saline suspension of liver, and 1 with 1 cc. of a 15 per cent saline suspension of spleen. The inoculum, in each instance, was rich in virus, as indicated by the presence of large numbers of inclusions. All 3 of the puppies died in from 36 to 48 hours after inoculation and at autopsy all showed increased vascular lesions in the brain and cord, characterized by proliferation of endothelial cells and innumerable minute hemorrhages. Nuclear inclusions were abundant in endothelial cells of the capillaries of the brain, spinal cord, lung, in reticulo-endothelial cells of the spleen, mesenteric lymph nodes, glomerular tufts of the kidney and in Kupffer cells and liver cells. These inclusions were also found in cells of the endocardium and in cells lining large vessels in the lungs, and in some instances in large mononuclear cells (not desquamated endothelium) lying free in large blood spaces in the adrenal and elsewhere. No inclusions were present in bronchial or bile duct epithelium. In each case there was a considerable amount of clear fluid in the peritoneal cavity, but inclusions were not detected in the serosal cells of the peritoneum. Sections of bone marrow from the femur of these animals showed extensive necrosis with great proliferation of the reticulo-endothelial cells and a corresponding diminution of red blood cell elements. The destructive action of the virus on megakaryocytes was particularly notable.

Each of 2 full-grown dogs was given an intravenous injection of 4 cc. of a 15 per cent saline suspension of liver tissue that contained many nuclear inclusions in liver cells and Kupffer cells. They were observed for a period of 10 weeks and at no time did they show evidences of infection. It was concluded that these animals were immune to distemper because of previous natural infection.

*Intracerebral Injections*

Three puppies were inoculated intracranially with 1 cc. amounts of a 15 per cent suspension of liver rich in virus. All died approximately 48 hours later. At autopsy the histological and cytological changes observed were identical with those seen in dogs that re-

ceived large doses of virus intravenously. In addition, the meninges of these animals were heavily infiltrated with mononuclear cells, many of which contained nuclear inclusions. In these cases nuclear inclusions were observed also in the ependymal cells lining the ventricles of the brain.

### *Intraneural Injections*

In an attempt to determine whether or not the virus reaches the central nervous system along nerve pathways, a sciatic nerve of each of 2 puppies was exposed and inoculated with a small amount of a saline suspension of liver that contained nuclear inclusions in large numbers. In each instance while the injection was being made a small amount of the inoculum escaped into the tissues surrounding the nerve. The operative wounds healed rapidly but both animals died of distemper 18 days later without showing symptoms referable to the nervous system. In each case sections of the injected nerve, dorsal root ganglia, spinal cord and brain failed to reveal any evidence of virus activity. Nuclear inclusions, however, were found in endothelial cells of the spleen and mesenteric lymph nodes.

### *Intratracheal and Intranasal Inoculations*

Three puppies were inoculated intratracheally with the virus. In another case the virus was instilled into the nose of a puppy. All died within 15 to 21 days after the inoculations. The microscopic pathology in these cases was identical with that already described in the young dogs of Group I that died of the natural disease. In each case there was a definite interstitial pneumonia, and both nuclear and cytoplasmic inclusions were numerous in the bronchial epithelium. Purulent bronchopneumonia did not occur.

### *Subcutaneous and Intradermal Inoculations*

One puppy was inoculated subcutaneously in the abdominal wall with 1 cc. of a 15 per cent saline suspension of liver tissue rich in virus. The following day the entire anterior abdominal wall was edematous and somewhat reddened. The edema and redness persisted until the animal died 3 days later. At autopsy the tissues about the injected site were heavily infiltrated with polymorphonu-

clear leukocytes and mononuclear cells, the latter of which, in many instances, contained nuclear inclusions. There were small areas of focal necrosis in the liver and many nuclear inclusions were present in liver cells, Kupffer cells, and in endothelial cells of the spleen, mesenteric lymph nodes and lung capillaries.

Another puppy was given multiple intradermal injections of a similar suspension in the abdominal skin. Twenty-two hours later, when the injected sites appeared swollen and slightly ulcerated, one of the lesions was excised for histological examination. It showed a small abscess containing polymorphonuclear leukocytes, mononuclear cells and débris. The underlying dermis was edematous and invaded by mononuclear cells, in some of which nuclear inclusions were present. In addition the endothelial cells lining the lymphatics were swollen and often contained nuclear inclusions. Another lesion removed 48 hours after inoculation showed a larger area of necrosis, which extended well down into the dermis but otherwise appeared similar to the earlier lesion. This animal died 25 days after inoculation. Autopsy revealed areas of focal necrosis in the liver, but inclusions were found only within nuclei of endothelial cells of the spleen and mesenteric nodes.

In another case virus-containing liver was thoroughly rubbed into a small scarified area of the skin of a young puppy. The inoculated area became reddened and slightly edematous and exuded serum for a few days. This animal never appeared definitely ill. Approximately 2 months after the skin inoculation it was given an intravenous injection of 4 cc. of a saline suspension of liver rich in virus. The animal proved to be immune.

### *Feeding Experiments*

Each of 2 puppies was fed a portion of milk containing 10 cc. of a thick suspension of virus-containing liver. One died 9 days, and the other 10 days later. Autopsy revealed many small hemorrhages in the mucosa of the intestine and colon and sections of these organs revealed the submucosa to be infiltrated heavily with mononuclear cells, many of which contained nuclear inclusions. Similar inclusions were detected in small numbers in glandular epithelium of the stomach and intestine. Nuclear inclusions were present in unusual abundance in reticulo-endothelial cells of the mesenteric lymph

nodes, in less abundance in reticulo-endothelial cells of the spleen, and were rare in Kupffer and liver cells.

### *Filtration Experiments*

Six animals, including 2 controls, were used in filtration experiments. In one experiment a 15 per cent saline suspension of virus-containing liver was centrifuged at high speed for 15 minutes. The supernatant fluid was passed through a Berkefeld N filter. The filtrate, proved by cultures to be bacteria-free, was injected intravenously into 2 puppies, 1 being given 5 cc., the other, 10 cc. Another puppy was injected intravenously with 1 cc. of the original liver suspension. The control animal died 3 days later and at autopsy presented the findings already described in cases where the virus was injected intravenously in large amounts. The puppy that received 5 cc. of the filtrate was killed while in a moribund state 15 days after inoculation. Microscopic sections revealed nuclear inclusions, somewhat smaller than those usually seen, in liver cells and Kupffer cells. Similar inclusions were also present in reticulo-endothelial cells of the spleen and mesenteric lymph nodes. There was a lesion in the brain involving Ammon's horn and the basis pedunculae of the mesencephalon on one side. The histological features of this lesion were similar to those already described under the subheading "intravenous injections" as occurring in the puppy that received an intravenous injection of 5 cc. of blood obtained from this animal. In this lesion both cytoplasmic and nuclear inclusions were unusually prominent in neuroglia, microglia and nerve cells. In some nerve cells, usually in association with nuclear inclusions, cytoplasmic inclusions were most numerous, sometimes as many as 20 being seen in a single cell. They appeared as vacuolated, acidophilic bodies of varying size often undergoing fusion to form larger bodies of irregular but sharply defined outline. Many appeared indistinguishable from Negri bodies. But the presence of associated nuclear inclusions, usually in the same nerve cells, and also in glial cells, served to differentiate the lesion from rabies.

In the second filtration experiment peritoneal fluid fro n a puppy that died after receiving an intraperitoneal injection of a large amount of virus was centrifuged at high speed for 15 minutes. The supernatant fluid was passed through a Berkefeld N filter. Six cc.

of this filtrate, proved by culture to be bacteria-free, was injected intravenously into a puppy. The animal died 3 days later and, as in the case of a control puppy inoculated intravenously with a 3 cc. portion of the unfiltered peritoneal fluid, the autopsy findings were identical with those already described as occurring in dogs and puppies that died after being injected intravenously with a large amount of virus. Another puppy was inoculated intracranially with 1.5 cc. of the filtrate. This animal took very little food and was definitely ill for a week after the inoculation, but it soon recovered and now, approximately 3 months after inoculation, appears normal.

### *Viability of the Virus*

As determined by intraperitoneal inoculation of puppies, the virus contained in liver tissue showed no appreciable deterioration after storage at a temperature of 10° C. for a period of 35 days. Such material, after storage at the same temperature for 85 days, was apparently non-infectious. The virus in liver tissue preserved in equal parts of glycerin and physiological salt solution and stored at 10° C. for 67 days, though partially deteriorated, proved capable of inducing the infection. Liver tissue containing the virus, after being dried *in vacuo* over phosphorus pentoxide, sealed in ampoules and stored at 10° C. for 90 days also proved infectious.

## Distemper in Ferrets

While our studies on distemper in ferrets have not, up to the present time, been extensive, we have made observations concerning the disease in this animal which we believe are worthy of record.

A fitch ferret was inoculated intraperitoneally with 2 cc. of a 15 per cent saline suspension of dog liver rich in distemper virus. During the following 2 weeks the animal appeared normal. It then became unusually drowsy and ate very little. On the 25th day after the inoculation it had a notable purulent conjunctivitis, the lips and foot-pads were swollen and crusty, and superficial ulcers were present in the abdominal skin. The animal then developed a flaccid paralysis in both lower extremities and died 4 days later. Autopsy revealed an extensive interstitial pneumonia, similar to that seen in dogs with the natural disease, and a superimposed purulent bronchopneumonia. Both nuclear and cytoplasmic inclusions occurred in

abundance in epithelial cells of the trachea, bronchi and pulmonary alveoli. The liver showed increased fatty degeneration but no necrosis. Inclusions were occasionally seen in the nuclei of liver cells and Kupffer cells, but the great majority of the epithelial cells of the bile ducts contained within their nuclei, single, irregularly shaped, homogeneous acidophilic inclusions, and from 1 to 3 sharply defined, usually round to oval, vacuolated eosinophilic inclusions in their cytoplasm. Both types of inclusions appeared similar to the corresponding types seen in bronchial and alveolar cells. Nuclear inclusions were also fairly abundant in endothelial cells of the spleen and swollen mesenteric lymph nodes. In sections of the brain, particularly those from Ammon's horn, nuclear and cytoplasmic inclusions similar to those encountered in the brains of some dogs were found. Sections of the tongue, lips, abdominal skin and foot-pads showed small intradermal abscesses filled mainly with polymorphonuclear leukocytes. Nuclear and cytoplasmic inclusions, such as those present in the epithelium of bronchi and bile ducts, occurred in the surrounding epithelial cells in large numbers, indicating a definite etiological relationship of the virus to these lesions.

Not realizing at the time the extreme susceptibility of the ferret to distemper, the single ferret inoculated was kept in a separate cage but in the same room with 5 other ferrets. About 6 days after the inoculated animal developed conjunctivitis and swelling of the lips, 4 of the 5 stock ferrets developed similar symptoms and all died within 6 to 8 days without showing symptoms referable to the central nervous system. The 5th animal failed to contract the disease. Autopsy of the animals that died of the contact infection revealed findings similar to those encountered in the inoculated animal, with the exception that there were no definite lesions in the brain and no inclusions in the nuclei of the liver cells or Kupffer cells.

## Susceptibility of the Chorio-allantoic Membrane of Chick Embryos to the Virus

In preliminary experiments we have secured evidence that the chorio-allantoic membrane of chick embryos can be infected with the virus of distemper. In microscopic sections of membranes of 12 day old embryos obtained 48 hours after their inoculation with virus-containing liver we have observed evidence of virus activity, as

indicated by nuclear inclusions in ectodermal cells and in large mononuclear cells infiltrating the mesodermal layer. Our studies in this connection are not as yet extensive enough to enable us to give a detailed description of these lesions. Also, further studies are required to determine whether or not the virus can be propagated in series in this medium.

## Discussion

The series of young puppies that died of naturally acquired distemper included cases with and without symptoms referable to the central nervous system. In every animal of this series (13 in number) lesions were observed in the lungs, spleen and mesenteric lymph nodes, which we regard as characteristic of distemper. In the lungs there is an interstitial pneumonia, such as that produced by a variety of viruses, in which the alveolar walls and peribronchial tissues are infiltrated with large mononuclear cells. Desquamation of epithelium of the smaller bronchi is usually extensive. The specificity of this particular lesion is indicated by the presence of cytoplasmic and nuclear inclusions in the bronchial and alveolar epithelium. These inclusions have been observed in the experimental disease only in those cases where the virus was introduced by way of the respiratory tract. The constancy with which they occur in cases of the natural disease indicates that the common portal of entry is the respiratory tract. Because we succeeded in inducing the disease with intradermal and subcutaneous inoculations of the virus, and because the virus has been proved by other investigators to be present in the saliva of dogs with distemper, there may be occasional cases where the disease is transmitted to a susceptible animal by the bite of an infected dog.

In the spleen, in both the natural and the experimental disease, there is a follicular necrosis, proliferation of reticulo-endothelial cells and nuclear inclusions in some of these cells, usually in those within the follicles. The same type of inclusion occurs in reticulo-endothelial cells of the swollen mesenteric lymph nodes. In all cases of the natural disease, except one, nuclear and cytoplasmic inclusions, similar to those occurring in bronchial epithelium, were found in bile duct epithelium. In 2 cases these inclusions were abundant, but in the others they were detected only after prolonged search. In the

experimental disease inclusions were not present in the bile duct epithelium.

In the case of the young puppy (Group III) that died of an acute fulminating type of the disease, and from which the strain of virus largely used in our experimental studies was obtained, the great affinity of the virus for vascular endothelium and cells of the reticulo-endothelial system is well demonstrated. Also, in this case most of the liver cells contained nuclear inclusions, the occurrence of which has not heretofore been reported in canine distemper. Cowdry and Scott,[23] however, described similar inclusions in liver cells and Kupffer cells in 2 of Dieckmann's experimental dogs in which distemper was not suspected. It seems probable, in the light of our observations, that these investigators were dealing with dogs suffering from distemper. The microscopic pathology of our case under discussion was quite similar to that of the so-called "epizoötic fox encephalitis" of Green, as described by Green, Ziegler, Green and Dewey.[24] These investigators did not mention the occurrence of nuclear and cytoplasmic inclusions in bronchial and pulmonary alveolar cells which we have constantly observed in dogs dying from naturally acquired distemper. Lately, in a further study of the fox disease, Green and his co-workers[25] have observed nuclear inclusions, but not cytoplasmic inclusions, in epithelial cells of the upper respiratory tract of foxes dying from the natural disease. In cases where the disease was induced in foxes by closed injections of virus, these inclusions were detected only when the experimental disease ran a prolonged course. Green and Shillinger[26] transmitted the fox disease to dogs and expressed the opinion that the disease probably occurs naturally in these animals. Green and Dewey[27] could not infect ferrets with the virus of fox encephalitis but, like Dunkin and Laidlaw,[3] they found these animals highly susceptible to canine distemper. They found that injections of fox encephalitis virus into ferrets gave no immunity against subsequent injections of canine distemper virus. The single ferret that was injected with our strain of virus became sick after a rather prolonged incubation period and the possibility of a spontaneous infection cannot be ruled out. With the exception of the abscesses in the lips, tongue, abdominal wall and foot-pads, the histological and cytological features presented by this animal have all been observed by us in dogs that died of experimental distemper. Among these was the brain lesion characterized by degeneration of

neurons and the presence of both nuclear and cytoplasmic inclusions in neurons and glial cells. Brain lesions were not observed in the 4 ferrets that developed the disease by contact, but otherwise the autopsy findings in these animals were identical with those observed in the experimental animal.

The virus of distemper is evidently cytotropic and cells derived from all three germinal layers are susceptible to it, judging from the presence in them of inclusions. The extensive involvement of endothelium indicates that the virus spreads in the tissues of its host mainly by way of the blood stream. Attempts to demonstrate its passage along nerve pathways have failed. In the brain lesions found in 3 cases of the natural disease, in 2 cases of the experimental disease in puppies, and in 1 ferret inoculated with the virus, there were persistent vascular lesions characterized by proliferation of endothelium and perivascular mononuclear cell infiltration with both nuclear and cytoplasmic inclusions in neurons and glial cells. In many instances the cytoplasmic inclusions appeared indistinguishable from Negri bodies. The presence of the associated nuclear inclusions offers a better basis of differentiation between the two than differences in morphology and staining reactions. Lesions of this type occurring in the brains of some dogs in the late stages of the disease undoubtedly arise secondarily to the earlier, acute vascular lesions, and this probably accounts for their varied distribution in different cases. Because the highly specialized cells of the brain are less frequently affected by the virus they are presumably not involved as quickly as other cells, such as those of endothelium, and it seems that the nervous type of lesion is to be expected only in those animals whose immunological response to the virus is rather slow. Lesions of this type were probably present in the brains of all 5 of the dogs that died of the natural disease with symptoms of the nervous type of distemper, but in 2 instances they escaped detection.

Several clinical types of distemper have been described, such as catarrhal, nervous or encephalitic, intestinal and tegumentary. We have described another type which we propose to call the acute encephalitic or, more properly, the acute encephalomyelitic type, which is a rapidly fulminating form of the disease characterized by extensive involvement of vascular endothelium by the virus, particularly that of the brain and cord, and of reticulo-endothelial cells and liver cells. This type of the disease may be fairly common

in very young puppies but passes unrecognized, perhaps, because it runs such a short course.

Some investigators are of the opinion that different strains of virus are responsible for the various clinical types of the disease. Judging from our observations in the study of both the natural and the experimental disease, the catarrhal features are present in every case, but in many cases are overshadowed by more outstanding symptoms referable to some system other than respiratory. With a single strain of virus we have reproduced the catarrhal form of the disease in some animals, the well known nervous type in others, and in still others the less well known acute encephalitic type. Also, in the gastro-intestinal tracts, particularly in the colon of some of the experimental animals, there were definite evidences of virus activity, a feature we believe is responsible for the diarrhea, a predominant symptom in dogs suffering from the intestinal form of the disease. Abscesses of the abdominal wall did not occur in our experimental puppies and they were seldom observed in the animals that died from the natural disease. Because abscesses of the lips, tongue and abdominal wall were an outstanding clinical feature of the disease in ferrets, and since both nuclear and cytoplasmic inclusions occurred in abundance in the epithelium adjacent to these abscesses, we feel sure that these lesions, as well as similar lesions in the dog, when they occur, are initiated by the virus.

The virus of canine distemper is one of the few viruses capable of producing both cytoplasmic and nuclear inclusions. Others known to be capable of bringing about a response on the part of both nucleus and cytoplasm are those of smallpox (Councilman, Magrath, and Brinckerhoff [28]), paravaccinia (Lipschütz [29]), and alastrim (Torres and Teixeira [30]), all of which are regarded by some investigators as being closely related or possibly modified forms of the same virus.

The nuclear inclusions of distemper, like those occurring in many other virus diseases, are acidophilic homogeneous bodies. The cytoplasmic inclusions are also acidophilic but contain well defined vacuoles and, as emphasized by Golgi and Sinigaglia, who demonstrated inner structures within them, they are indistinguishable from Negri bodies on the basis of morphology and staining reactions. In the brain, however, the lesions of the two diseases may be differentiated histologically by the presence of associated nuclear inclusions

and by the absence of extensive inflammatory reaction in distemper. As Negri bodies are known to occur only in nerve cells, cytoplasmic inclusions occurring in other types of cells should not cause confusion.

## Summary and Conclusions

1. The most important literature on canine distemper is reviewed.

2. In a histological and cytological study of both the natural and the experimental disease in puppies, lesions that we believe to be characteristic of the disease are described.

3. The virus of canine distemper has a definite affinity for vascular endothelium and for cells of the reticulo-endothelial system.

4. The virus spreads in the body of the host mainly by way of the blood stream. We have obtained no evidence that it passes along the nerve pathways.

5. The natural route of infection is by way of the respiratory tract.

6. The occurrence of nuclear inclusions, heretofore unreported, in liver cells, bronchial epithelial cells, glandular cells of the stomach and intestine, and bile duct epithelial cells, and cytoplasmic inclusions in bile duct epithelial cells are described.

7. A heretofore unreported clinical type of the disease with a characteristic microscopic pathology is described and has been reproduced in puppies.

8. Various clinical types of the disease have been induced in puppies with a single strain of virus.

9. The histopathology and cytology of the disease in dogs and ferrets are quite similar.

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## DESCRIPTION OF PLATES

### PLATE 27

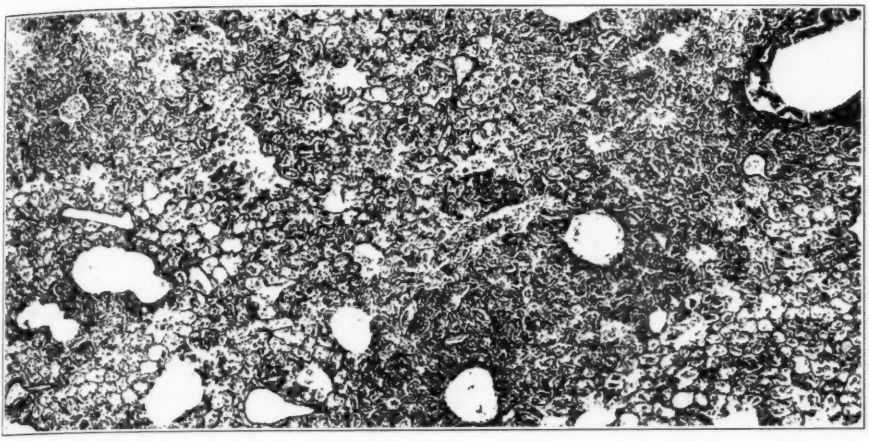
FIG. 1. Section of lung of dog that died of naturally acquired distemper. Note the interstitial pneumonia. Epithelium of smaller bronchi is desquamated. Hematoxylin and eosin stain. × 46.

FIG. 2. Bronchial epithelium in section of lung shown in Fig. 1. Note the vacuolated, cytoplasmic inclusions. Hematoxylin and eosin stain. × 1400.

FIG. 3. Same as Fig. 2, but a nuclear and cytoplasmic inclusion within the same cell. Hematoxylin and eosin stain. × 1400.

FIG. 4. Section of spleen of dog that died of naturally acquired distemper. Note necrosis within follicles and proliferation of mononuclear cells. This lesion occurred constantly in both the experimental and the natural infection. Hematoxylin and eosin stain. × 46.

FIG. 5. Nuclear inclusion in reticulo-endothelial cell of spleen shown in Fig. 4. Hematoxylin and eosin stain. × 1400.



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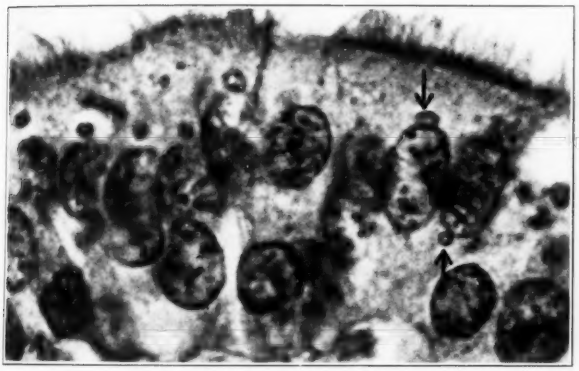

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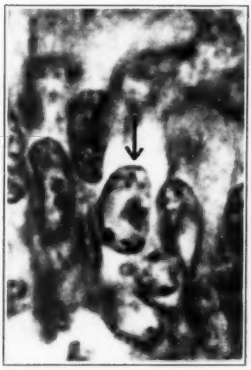

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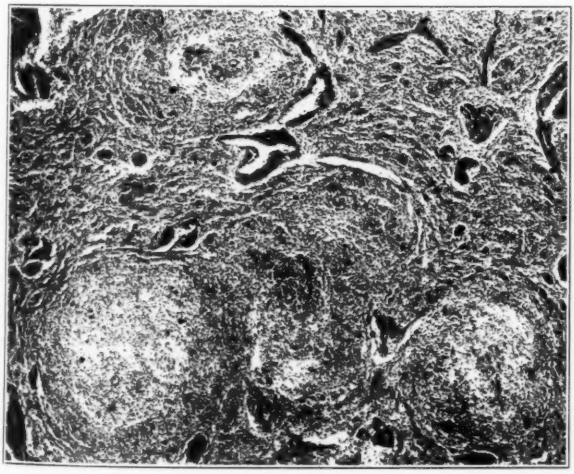

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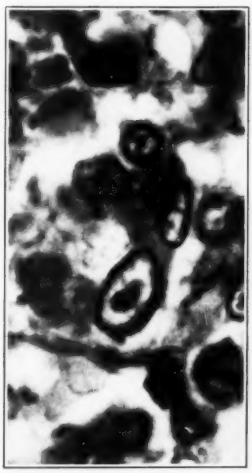

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DeMonbreun Histopathology of Canine Distemper

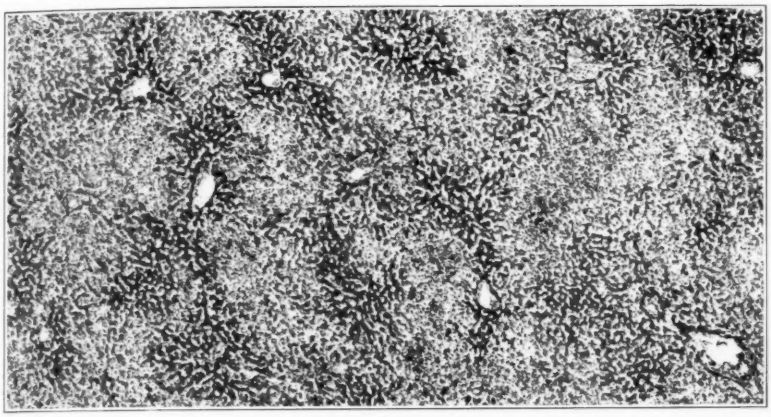
PLATE 28

FIG. 6. Section of liver of puppy that died 8 days after receiving an intraperitoneal injection of virus. Note the extensive areas of focal necrosis which contain many large mononuclear cells. Examination under high power lens reveals only an occasional inclusion in liver and Kupffer cells. Hematoxylin and eosin stain × 46.

FIG. 7. Section of liver of puppy that died approximately 72 hours after receiving an intraperitoneal injection of virus. Almost every liver cell contains a nuclear inclusion. One Kupffer cell containing a nuclear inclusion is seen at the edge of a sinusoid half-way between the center and right border of the photograph. There is remarkably little necrosis. Hematoxylin and eosin stain. × 750.

FIG. 8. Section of colon of a dog that died of the natural infection. Note the nuclear inclusion in one glandular cell of the mucosa. Hematoxylin and eosin stain. × 1400.

FIG. 9. Section of skin removed from puppy 22 hours after intradermal inoculation of virus. Note nuclear inclusions in endothelial cells of lymphatics. Hematoxylin and eosin stain. × 1400.



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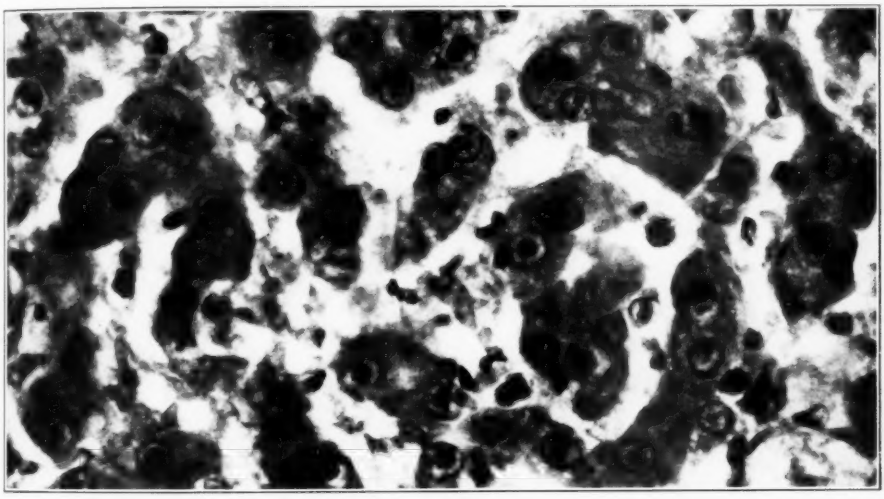

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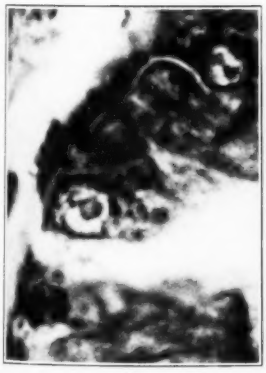

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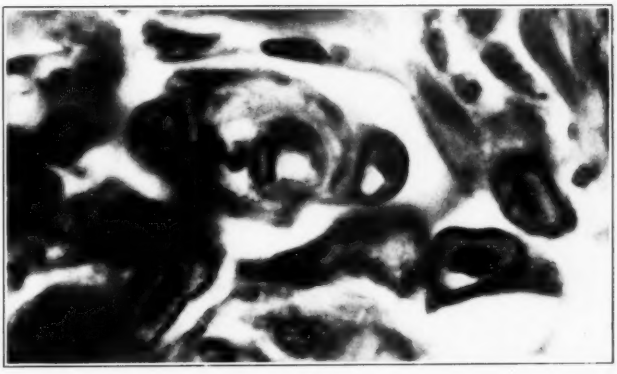

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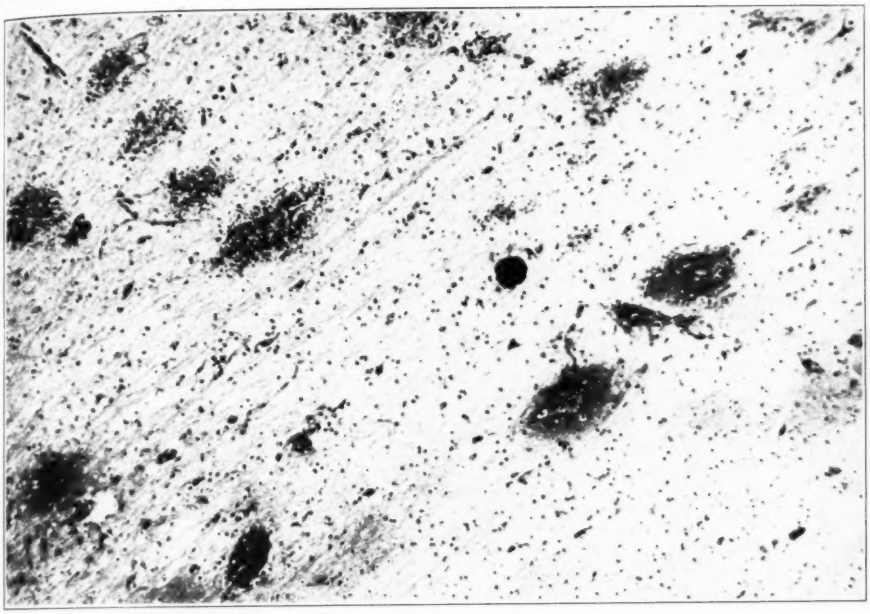
## PLATE 29

FIG. 10 Section of brain of puppy that died approximately 48 hours after receiving an intravenous injection of distemper virus. Note proliferation of vascular endothelium and multiple small hemorrhages. Section of brain of 1 puppy that died of the natural infection and which was the source of the strain of virus largely used in our experiments showed similar lesions. Hematoxylin and eosin stain. × 46.

FIG. 11. Higher magnification of capillary shown near center of Fig. 10. Note the nuclear inclusion in one of the swollen endothelial cells and the extravasated blood in the Virchow-Robin space. Hematoxylin and eosin stain. × 1400.

FIG. 12. Another longitudinally cut capillary from section shown in Fig. 10. Two endothelial cells contain well defined inclusions. Hematoxylin and eosin stain. × 1400.



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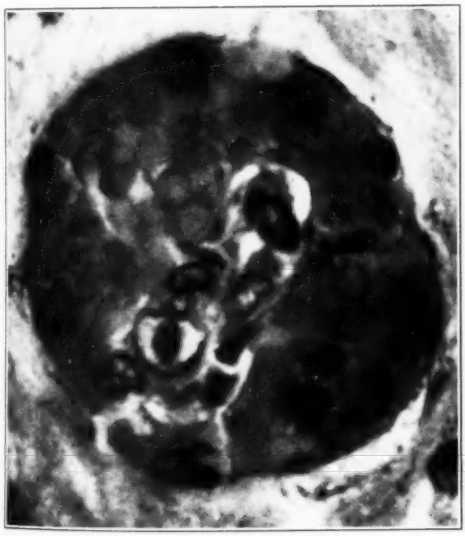

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12

DeMonbreun

Histopathology of Canine Distemper

PLATE 30

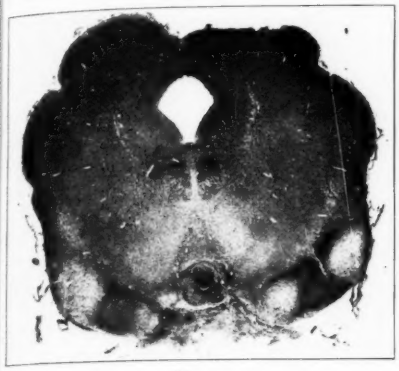
FIG. 13. Section taken from the mesencephalon of a puppy that died 72 days after receiving an intraperitoneal injection of 5 cc. of blood from a moribund puppy that had, 15 days previously, been injected with a Berkefeld filtrate of liver tissue containing numerous nuclear inclusions. Note the lesions which appear as light areas in each basis pedunculae. Phosphotungstic acid hematoxylin stain. × 4.

FIG. 14. Higher magnification of one of the lesions shown in Fig. 13. Note the nuclear inclusions in glial cells. Hematoxylin and eosin stain. × 1400.

FIG. 15. Section from Ammon's horn of another experimental dog that died of distemper of the nervous type. Three of the neurons contain nuclear inclusions. Cytoplasmic inclusions are also present in these cells but they are shown very poorly by this stain. Hematoxylin and eosin stain. × 1400.

FIG. 16. Section from the same Ammon's horn showing well defined nuclear and cytoplasmic inclusions in three cells. The cytoplasmic inclusions are vacuolated and appear quite similar to Negri bodies. Goodpasture's carbol aniline fuchsin stain. × 1400.

FIG. 17. Another neuron from the same Ammon's horn. The cytoplasmic inclusions have fused to form an irregularly shaped vacuolated mass. Goodpasture's carbol aniline fuchsin stain. × 1400.



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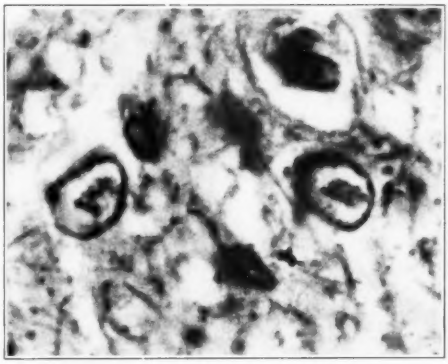

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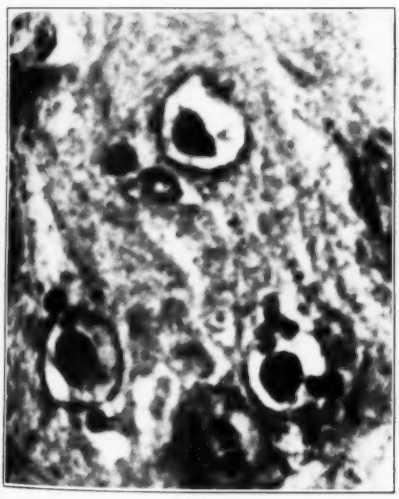

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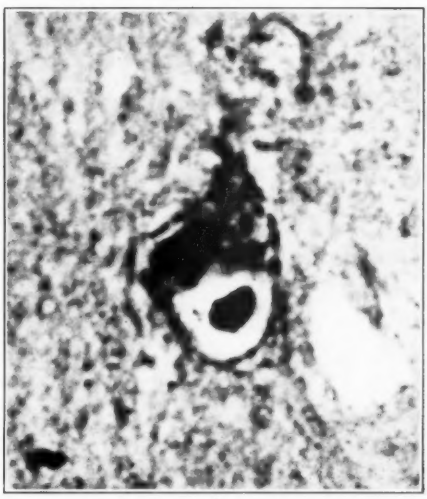

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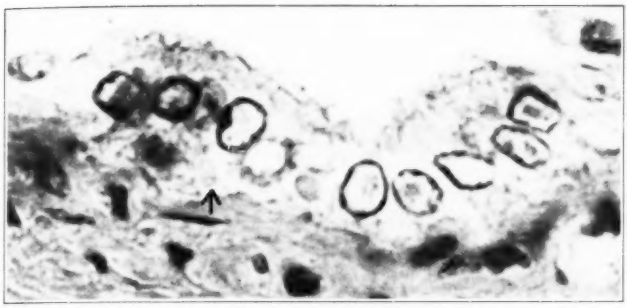
PLATE 31

FIG. 18. Section of bile duct of ferret showing both nuclear and cytoplasmic inclusions in bile duct epithelium. The arrow points to a cytoplasmic inclusion. The nuclear inclusions are quite apparent. Hematoxylin and eosin stain. × 1400.

FIG. 19. Section of tongue of ferret showing both nuclear (arrow) and cytoplasmic inclusions in epithelial cells. Similar inclusions were also observed in the epithelium of the lips and abdominal skin. Hematoxylin and eosin stain. × 1400.

FIG. 20. Well defined cytoplasmic inclusions in epithelial cells of the abdominal wall of a ferret. Hematoxylin and eosin stain. × 1400.



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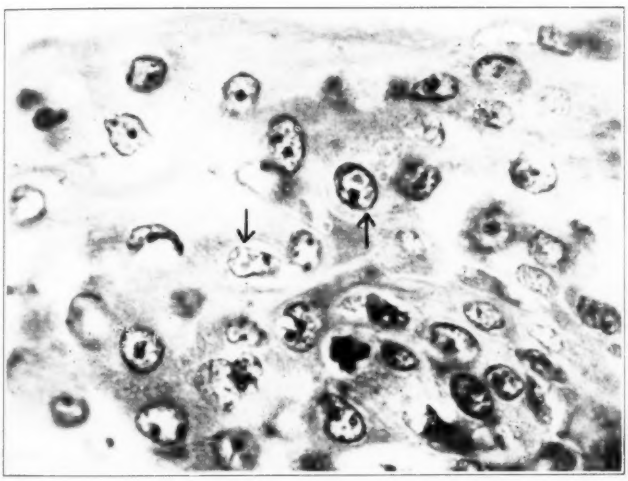

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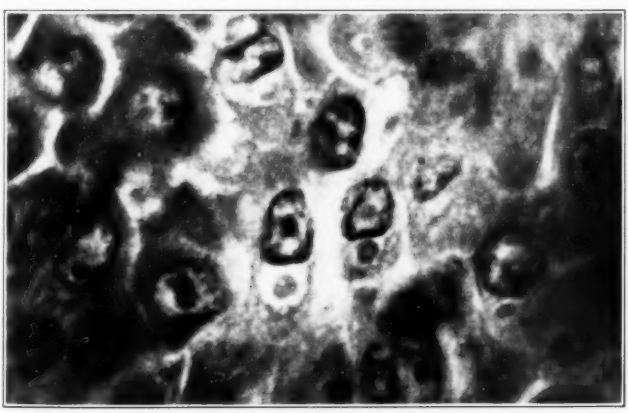

20

DeMonbreun Histopathology of Canine Distemper

# BASOPHIL INFILTRATION IN THE NEUROHYPOPHYSIS *

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Although basophilic infiltration of the posterior lobe of the pituitary body has been observed and commented on for many years, Cushing[14] was the first to emphasize it as a pathological process and to attempt to relate it causally to hypertensive states. Thom[39] is generally agreed to have been the first to describe it, in a female of 90 years whose final diagnosis was *marasmus senilis and bronchitis.* Although he gives details of only 9 of his 62 cases, he remarks that such invasion is often seen after the end of the third and beginning of the fourth decade. He believed this in-wandering to be a physiological process, associated with advancing age, and representing the customary mode of obliteration of the hypophyseal cleft. Erdheim[15] observed it three times in 50 cases and agreed with Thom's interpretation. He noted that the basophils seen in the posterior lobe were relatively poor in fat and therefore deduced that they were young cells. Löwenstein[28] confirmed these findings. Herring[22] saw the same phenomenon in the pituitary of the cat and associated the presence of these cells with the production of the colloid which he observed in large quantities in the posterior lobe. He believed the source of the cells to be the pars intermedia.

Lucien[29] objected to the idea implied in Thom's description, that such an invasion might be considered a species of tumor. He remarked that invasion was seen in the majority of elderly people and was usually accompanied by an increase in the basophils of the anterior lobe. Erdheim and Stumme[17] could find no noticeable difference in this respect between the normal hypophysis and the hypophysis of pregnancy.

Tölken[40] in a careful study of 105 pituitaries observed basophilic infiltration in 50; the majority were from patients over 40 years,

* This study was aided by a grant from the research funds of the Yale University School of Medicine.

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although the youngest was 18. He concluded that these cells arose from the margins of the hypophyseal cleft or its remains. He could demonstrate no correlation between the degree of invasion and sex or disease.

Vogel,[41] in a study of the pigment occurring in the posterior lobe, demonstrated basophilic invasion in 87 cases out of 102, ranging in age from newborn to 90 years. His youngest positive case was a 9 months old child. He believed that the basophils represented a system of transport of anterior lobe secretion to the brain, and that the pigment of the posterior lobe was the waste product of their breakdown. He agreed with Tölken that sex and disease bore no relation to the infiltration.

Berblinger[3] and Höppli[23] examined 45 cases of kidney disease (all kinds) and 75 controls. They concluded that there was a definite increase in anterior lobe basophils in renal disease, but could demonstrate no difference in the degree of posterior lobe infiltration from that found in their controls and agreed upon in the literature as normal. Kraus[24] commented on the lack of a quantitative relation between anterior and posterior lobe basophils and suggested this as evidence that they had different functions. Skubiszewski,[35] in a comparison of the pituitaries of 23 cases of *nephritis interstitialis genuina* and 75 miscellaneous controls, confirmed the findings of Berblinger and Höppli that the basophils of the anterior lobe were increased in renal disease, and also thought that the degree of invasion of the posterior lobe was considerably greater in this than in any other condition. He related the polyuria and left ventricular hypertrophy of *nephritis interstitialis genuina* to the increased basophilia of both anterior and posterior lobes, and suggested that the function of these cells was to cause a rise in blood pressure and dilatation of the renal vessels.

Erdheim[16] emphasized the point that there is no true pars intermedia in man, a statement which, as he remarked, was tantamount to sticking his head into a wasp's nest. The basophil cells of the posterior lobe arise in the anterior lobe and invade the posterior lobe by independent locomotion. The cells of the Rathke cysts, remains of the hypophyseal cleft, differentiate like the cells of the anterior lobe. The racemose glands so often seen in the boundary zone between the anterior and posterior lobes are true mucous glands, reminiscent of the origin of the hypophysis from the pharyngeal

epithelium. Guizzetti[19, 20] announced that there was definitely a pars intermedia in man and that the basophils seen in the posterior lobe took origin from the undifferentiated epithelial cells of the dorsal lip of the hypophyseal cleft. Invasion of the posterior lobe is seen in four-fifths of individuals past the age of 30 years and has certain sites of predilection, namely, midway between the cephalic and caudal boundaries of the posterior lobe and also at the extreme caudal boundary beneath the capsule. The cells invade the neural tissue not only by proliferation, but also, as Erdheim had said, by active motion. The degree of infiltration is not influenced by sex, nor by cardiopathies or chronic nephritides, but there is a definite increase in tuberculosis and in diseases affecting the cerebrospinal axis directly. Schönig[34] too maintained, on embryological and morphological grounds, that there was a pars intermedia in man. He confirmed the findings of other workers that there was an increase in posterior lobe basophilia with age and associated this with the increased connective tissue proliferation of the posterior wall of the hypophyseal cleft which is responsible for its division into numerous small cysts.

Kraus[25] and Berblinger[4] promptly took issue with Schönig's contentions, Kraus stating with some asperity that the "Markschicht" of the human hypophysis was properly part of the anterior lobe and not to be confused with the morphologically independent and distinguishable pars intermedia of animals. Berblinger further pointed out that both anterior lobe and pars intermedia arise from Rathke's pouch, and that in man no distinctive epithelial structure of a specific and different cell type, arising from the dorsal lip of the hypophyseal fissure, such as is seen in animals, is demonstrable. The Rathke cysts are part of the anterior lobe, and the cells lining them may differentiate into typical chromophils, as Höppli, Erdheim and others had previously shown. There are only two parts to the pituitary in man, the adenohypophysis and the neurohypophysis. Lewis and Lee[27] after a careful study of 30 pituitaries stated that two epithelial elements, racemose glands and basophil cells, were found at all ages in the posterior lobe, and these glandular elements should be included under the term *pars intermedia*. They could find no constancy in age incidence or position for either glands or basophils, although the basophils seemed to increase with advancing years.

Kraus and Traube,[26] having examined 232 pituitaries, concluded that the incidence of basophils in both anterior and posterior lobes was influenced by body build, being more notable in hypersthenic individuals, and also in renal disease, essential hypertension and a number of other conditions. Posterior lobe basophilia increased with age and was more prominent in males than in females. They repeated Kraus's former statement that there was no quantitative relation between anterior and posterior lobe basophilia and therefore the two sets of cells must have different functions. They advanced the interesting theory that the basophils of the anterior lobe act as a sort of check-rein on the blood pressure, and that they hypertrophy in an endeavor to overcome the increasing hypertension. Berblinger,[5] on the basis of 71 cases of renal disease and 71 controls, agreed with their statement that the anterior and posterior lobe basophils bear no relation to each other, and observed as he did in 1922 that the anterior lobe basophils are increased in number in renal disease. He disagreed completely with their view that there was any correlation between body build and basophilism.

Cushing[12] suggested that in basophil adenoma the secretion of the basophil cells might pass through the posterior lobe and stalk and exert a stimulatory effect on the diencephalic nuclei, thus leading to the adiposity, hypertension and vascular changes of this syndrome. In a second paper[13] he stated that the secretory product of the posterior lobe is unmistakably derived from the investing pars intermedia, whose cells become basophilic when ripened, are cast off and invade the pars nervosa. In certain disorders characterized notably by hypertension, but also by other symptoms suggestive of known physiological effects of posterior lobe secretion, the normal cellular activity of the pars intermedia becomes greatly exaggerated, as is shown by a marked hyperplasia of the basophil elements, which penetrate far into the posterior lobe.

Guizzetti[21] repeated his previous statements[19, 20] as to the origin and incidence of posterior lobe basophils and stated that they were as a rule smaller than those of the anterior lobe and contained no vacuoles, an observation previously made by Erdheim.[15] They were identical, however, in staining reactions.

Cushing,[14] on the basis of 9 cases of toxemia of pregnancy, 1 case of essential hypertension, and 3 cases of hypertension with atherosclerosis, arrived at the following conclusions: (1) that the source of

these hypertensive disorders (toxemia of pregnancy, essential hypertension) lies in the posterior lobe of the pituitary body; (2) that the extent of basophilic invasion from the pars intermedia is a measure of posterior lobe activity; and (3) that excessive infiltration by these elements represents the histopathological basis of eclampsia and essential hypertension in young persons and may possibly be related etiologically to the atherosclerosis of old age. These observations served as the starting point for a number of workers, and were greeted with considerable scepticism by several. Close[10] again raised the objection that invasion of the posterior lobe is relatively common, particularly in middle-aged subjects, and that an increased infiltration may be seen in individuals whose blood pressures have been normal at least for several months before death. Butt and Van Wart[9] found posterior lobe invasion in 83.6 per cent of 126 hypophyses from unselected cases of all ages. Spark,[36] in 70 persons with essential hypertension, 11 persons with evidence of antecedent hypertension, and 108 controls of the same age groups, could find no significant difference in the degree of basophilism between the hypertensives and the controls. He concluded that posterior lobe invasion was related to the aging of the organism and possibly to some sex factor as well. Ahlström,[1, 2] on the other hand, in a series of 25 hypertensives and 36 normals showed that basophil infiltration occurred more frequently in hypertensives than in non-hypertensives. Biggart,[6] after examination of a large group of hypophyses, concluded that basophil infiltration may be found in the absence of hypertension, eclampsia, or any advanced degree of arteriosclerosis and does not seem to be found more commonly in these conditions than in control glands of the same age periods. The lack of constancy of the infiltration in any given syndrome rendered its functional interpretation impossible. Parsons,[31] after careful study of 107 unselected pituitaries, was able to demonstrate a very strong statistical correlation between age and the degree of invasion of the posterior lobe, but no significant correlation of invasion and either systolic or diastolic blood pressure.

The study on which this paper is based was undertaken in the hope that further light might be thrown on these highly controversial questions. We do not intend to become embroiled in the argument that rages over the presence or absence of a true pars intermedia in man and over the origin of the basophil cells seen in the posterior

lobe. Suffice it to say that there are two schools of thought on the subject, Thom, Erdheim, Löwenstein, Höppli, Berblinger, Kraus and others representing one side, and Tölken, Lucien, Skubiszewski, Schönig, Lewis and Lee, Guizzetti, Cushing and their followers, the other. Both sides agree, however, that the basophil cells of the posterior lobe are morphologically indistinguishable from those of the anterior lobe by ordinary staining methods. MacCallum *et al.*,[30] have recently reported a method of differentiating the two by the use of copper hematoxylin which appears promising.

## Materials and Methods

One hundred fifty-one routine autopsies performed at the New Haven Hospital in the years 1934–1936 and 2 outside cases were studied. The cases were divided into hypertensive and non-hypertensive groups on the basis of clinical findings, blood pressure and cardiovascular-renal findings at autopsy. In some instances patients with normal blood pressure and otherwise unexplained moderate or advanced left ventricular hypertrophy, generalized arteriosclerosis or contracted kidneys, were rated as hypertensives, while patients with elevated blood pressure on their hospital admission and no cardiac hypertrophy, arteriosclerosis or contracted kidneys were classed as non-hypertensives.* Routine microscopic preparations of the various organs were examined. The pituitaries were fixed in formaldehyde, embedded in paraffin, cut at 6 $\mu$, and every tenth section was mounted and stained with hematoxylin and eosin. Occasional sections were stained with methyl blue -eosin (Mann technic) and by the Rasmussen[32] modification of the Mallory connective tissue stain. Special care was exercised in the examina-

* Fishberg[18] and Stieglitz[37] agree, largely on the basis of Symonds'[38] tables, that hypertension may be defined as existing whenever the systolic pressure is 150 mm. or more or the diastolic pressure is 100 mm. or more, or both. Symonds' tables are based on life insurance applicants and lump together all persons over the age of 60. Bowes[8] in a study of 150 men and women over 65 years of age determined that the systolic pressure shows a tendency to rise and may reach 160 or 170 mm., while the diastolic shows very little change. It is only fair to state that the majority of Bowes' patients showed a considerable degree of cardiovascular pathology. It has not been possible to find any other articles discussing the question of blood pressure in the aged with more accuracy. For the purposes of this paper, a systolic blood pressure of 160 mm. in a patient over 60 years of age in the absence of left ventricular hypertrophy was not considered hypertension, but in all other respects the definition of hypertension given above was accepted.

tion of the neurohypophysis for basophilia, and the findings were recorded as follows:

– no basophil cells in the posterior lobe (Fig. 1).

± under 12 basophil cells, not clumped, in any one section.

+ slight basophilia consisting of a single clump of 12 or more cells, or several small clumps, or scattered individual cells more than 12 in number (Fig. 2).

++ one-fourth the posterior lobe invaded with basophil cells (Fig. 3).

+++ more than one-fourth the posterior lobe invaded with basophil cells (Fig. 4).

All of the pituitary sections were examined by three independent observers and their combined ratings were employed in the final grading.

## Results

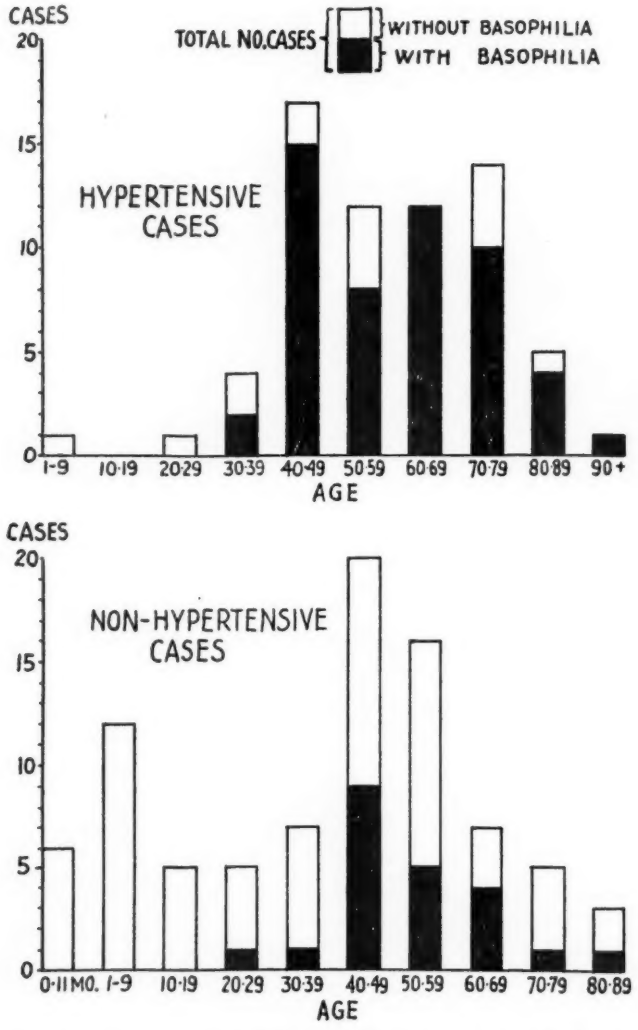
In Table I is recorded the degree of basophilic infiltration in the neurohypophyses of the hypertensive cases. The patients are divided into age groups by decades and according to sex. In the hypertensive group 60 of 67 patients showed some degree of basophilia in the neurohypophysis; 52 of 67 showed + or more; 29 of 67 showed ++ or +++; of these 29, 19 were males and 10 were females.

Similar data are presented for the non-hypertensive group in Table II. In this group 39 of 86 patients showed some degree of basophilia; 22 of 86 showed + or more; 6 of 86 showed ++, and all of these were males.

If all cases under 20 years of age in the non-hypertensive group are subtracted 22 of 63 showed + or more; 6 of 63 showed ++.

The graphs (Text-Fig. 1) show the relative incidence of basophilia in the hypertensive and non-hypertensive groups of cases. Here the fact is demonstrated clearly that significant basophilic infiltration (+ or more) is much more common in the hypertensives than in the non-hypertensives.

In Table III is presented the incidence of adenomas in the pars anterior of the pituitary bodies in this whole series.




TEXT-FIG. 1. — Showing relative incidence of basophilia in hypertensive and non-hypertensive groups of cases.

TABLE I

*Incidence of Basophilia in Neurohypophyses of Hypertensives*

| Age by decades | o | | ± | | + | | ++ | | +++ | | Total | | Grand total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | M | F | M | F | M | F | M | F | M | F | M | F | |
| *mos.* | | | | | | | | | | | | | |
| 0–11 | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | 0 | 0 | 0 |
| *yrs.* | | | | | | | | | | | | | |
| 1–9 | .. | .. | .. | 1 | .. | .. | .. | .. | .. | .. | .. | 1 | 1 |
| 10–19 | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | 0 | 0 | 0 |
| 20–29 | .. | .. | .. | 1* | .. | .. | .. | .. | .. | .. | 0 | 1 | 1 |
| 30–39 | .. | 1 | .. | 1 | 1 | .. | 1 | .. | .. | .. | 2 | 2 | 4 |
| 40–49 | .. | 2 | .. | .. | 3 | 3 | 4 | 3† | 2 | .. | 9 | 8 | 17 |
| 50–59 | .. | 2 | 1 | 1 | 3 | 2 | .. | 1 | 1 | 1 | 5 | 7 | 12 |
| 60–69 | .. | .. | .. | .. | 1 | 3 | 5 | 2 | 1 | .. | 7 | 5 | 12 |
| 70–79 | 1 | 1 | 2 | .. | .. | 4 | 3 | 2 | .. | 1 | 6 | 8 | 14 |
| 80–89 | .. | .. | .. | 1 | 1 | 2 | 1 | .. | .. | .. | 2 | 3 | 5 |
| 90–99 | .. | .. | .. | .. | .. | .. | 1 | .. | .. | .. | 1 | 0 | 1 |
| Total | 1 | 6 | 3 | 5 | 9 | 14 | 15 | 8 | 4 | 2 | 32 | 35 | 67 |

* Basophil adenoma
† One basophil adenoma.

TABLE II

*Incidence of Basophilia in Neurohypophyses of Non-hypertensives*

| Age by decades | o | | ± | | + | | ++ | | +++ | | Total | | Grand total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | M | F | M | F | M | F | M | F | M | F | M | F | |
| *mos.* | | | | | | | | | | | | | |
| 0–11 | 5 | 1 | .. | .. | .. | .. | .. | .. | .. | .. | 5 | 1 | 6 |
| *yrs.* | | | | | | | | | | | | | |
| 1–9 | 7 | 5 | .. | .. | .. | .. | .. | .. | .. | .. | 7 | 5 | 12 |
| 10–19 | .. | 4 | .. | 1 | .. | .. | .. | .. | .. | .. | 0 | 5 | 5 |
| 20–29 | .. | 1 | 1 | 2 | .. | .. | 1 | .. | .. | .. | 2 | 3 | 5 |
| 30–39 | 1 | 3 | .. | 2 | .. | 1 | .. | .. | .. | .. | 1 | 6 | 7 |
| 40–49 | 4 | 4 | 1 | 2 | 5 | 2 | 2 | .. | .. | .. | 12 | 8 | 20 |
| 50–59 | 7 | 1 | 1 | 2 | .. | 3 | 2 | .. | .. | .. | 10 | 6 | 16 |
| 60–69 | .. | 1 | 2 | .. | 2 | 1 | 1 | .. | .. | .. | 5 | 2 | 7 |
| 70–79 | 1 | 1 | 2 | .. | 1 | .. | .. | .. | .. | .. | 4 | 1 | 5 |
| 80–89 | 1 | .. | 1 | .. | 1 | .. | .. | .. | .. | .. | 3 | 0 | 3 |
| 90–99 | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | 0 | 0 | 0 |
| Total | 26 | 21 | 8 | 9 | 9 | 7 | 6 | 0 | 0 | 0 | 49 | 37 | 86 |

TABLE III

*Incidence of Pituitary Adenomas in Pars Anterior*

| Type | Hypertensive | | Non-hypertensive | | Total |
|---|---|---|---|---|---|
| | M | F | M | F | |
| Chromophobe | 4 | 2 | .. | .. | 6 |
| Eosinophil | .. | 1 | .. | .. | 1 |
| Basophil | 1 | 1 | .. | .. | 2 |
| Mixed (basophil and chromophobe) | 1 | .. | 1 | .. | 2 |
| Total | 6 | 4 | 1 | 0 | 11 |

## DISCUSSION

It will be observed from the preceding tables and the figures given above, that the incidence of posterior lobe basophilia appears to be strikingly higher in hypertensives than in non-hypertensives, and a severe grade appears to be more common in males than in females. It is impossible to state with any degree of accuracy that a severe grade occurs more frequently in any given age group, since it happens that in both series the age group with the highest incidence of severe basophilia is also the age group with the largest number of cases. It is, however, possible to state that in both series it appears after the age of 20 and is most common after the age of 40.

It seems reasonable to consider the ± groups as negative, since this designation usually implies a total of 60 cells or less scattered throughout the posterior lobe.

When the total 153 cases are examined, we find that 99 or 64.7 per cent show some degree of basophil invasion in the neurohypophysis, and if the ± groups are classed with the negatives, the figure becomes 74 or 48.3 per cent. Neither of these figures agrees with Butt and Van Wart's[9] finding of 83.6 per cent in 126 unselected cases of all ages.

If the 123 cases past the age of 30 are considered, it is found that 92 or 75 per cent show posterior lobe invasion (including those rated as ± ), a figure that corresponds fairly closely with that of 4/5 or 80 per cent given by Guizzetti.[20] If, however, the ± groups are lumped with the negatives, the figure falls to 73 or 59.1 per cent. It is a question whether or not it is entirely justifiable to compare this series of cases with those studied by Guizzetti, since 43.8 per cent of our series are of the hypertensive group, and his cases presumably,

although he gave no details as to the blood pressure, were individuals not chosen with such a classification in mind. If the non-hypertensives over the age of 30 alone are considered, 34 of 58 show some degree of basophilia, and 21 of 58 show + or more. To afford a basis of comparison, these figures may be translated as 60 per cent and 36.2 per cent respectively.

In other words, while basophil infiltration of the posterior lobe appears to be relatively common past the age of 20, it is not found in our series in such an overwhelming majority of the cases as in the other series mentioned above.

Since a marked degree (++ or +++) of infiltration is found in somewhat less than half the cases of hypertension, and is also found in 6 definitely non-hypertensive cases, it is difficult to demonstrate a relation between the degree of basophilia and hypertension. It even raises the question whether or not there is a causal relation between basophilia and hypertension. If such a relation exists, it is, as Russell, Evans and Crooke[33] have said of basophil adenoma, between basophil infiltration and high blood pressure as such, and not between basophil infiltration and chronic renal disease, arteriosclerosis or arteriolarsclerosis. In this connection it may be noted that 2 of our 3 cases of chronic nephritis with hypertension, both females, showed completely negative pituitaries, while the 3rd case, a male, showed a ++.

Only 1 case of "toxemia of pregnancy" is included in this series, and it is of interest that this patient, a negress 23 years of age, showed not only a basophil adenoma of the anterior lobe (unsuspected during life) but also extensive glomerular and tubular lesions in the kidneys.

When the 29 hypertensives with ++ or +++ invasion are examined with reference to body build, it is found that 23 of the 27 whose heights and weights were known were either within or below the average weight given for their height and age (Blanton[7]), allowing the usual leeway of 4.6 kg. on either side. Only 4 were above the average, and these ranged from 8.6 to 28 kg. above. Of these, 2 were males and 2 females. Among the 6 non-hypertensives only 1 was above the average in body build, and he was 13.6 kg. above. It should be pointed out that the majority of the cases were hospital patients who had been confined to bed for some time. The one hypersthenic non-hypertensive was a young male of 24 years who

was killed in an automobile accident. Thus we are unable to confirm Kraus and Traube's[26] finding that severe basophilism is more common in hypersthenic individuals.

Mixed basophil and chromophobe adenomas have only recently been described in the literature by Russell, Evans and Crooke,[33] Crooke,[11] and Parsons.[31] It is obvious that no conclusions can be drawn from so small a series, but it is perhaps of interest to emphasize the point brought out by Table III that pituitary adenomas of all sorts, in this series, are seen more commonly in the hypertensive than in the non-hypertensive group.

## Summary and Conclusions

1. One hundred fifty-three pituitaries have been studied in serial section. Both sexes and all age groups were represented, 67 hypertensives and 86 non-hypertensives.

2. Basophil infiltration of very slight (±) to advanced (+++) degree was seen in the posterior lobe in 64.7 per cent of all cases, including the hypertensive and non-hypertensive groups as well as all ages. Significant basophil infiltration (+ or more) was found in 52 of 67 hypertensives and in 22 of 86 non-hypertensives. Increased basophil infiltration (++ or more) was seen in 29 hypertensives, 19 males and 10 females, and in 6 non-hypertensives, all males. The only patients under 20 years to show any degree of infiltration at all (±) were a child of 6 with hypertension of unknown cause and a white female of 17 who died of bromide poisoning. Increased infiltration (++) was seen in only 2 cases under the age of 40.

3. It is concluded that significant basophil infiltration (+ or more) is much more common in hypertensives than in non-hypertensives, more common in the male than in the female, and more common after the 40th year.

4. Confirmation of Kraus and Traube's observation that severe basophil infiltration is more common in the hypersthenic individual could not be demonstrated.

5. Adenomas of the pituitary were seen in 10 hypertensives and in 1 non-hypertensive. Their type was as follows: chromophobe 6, eosinophil 1, basophil 2, and mixed basophil and chromophobe 2.

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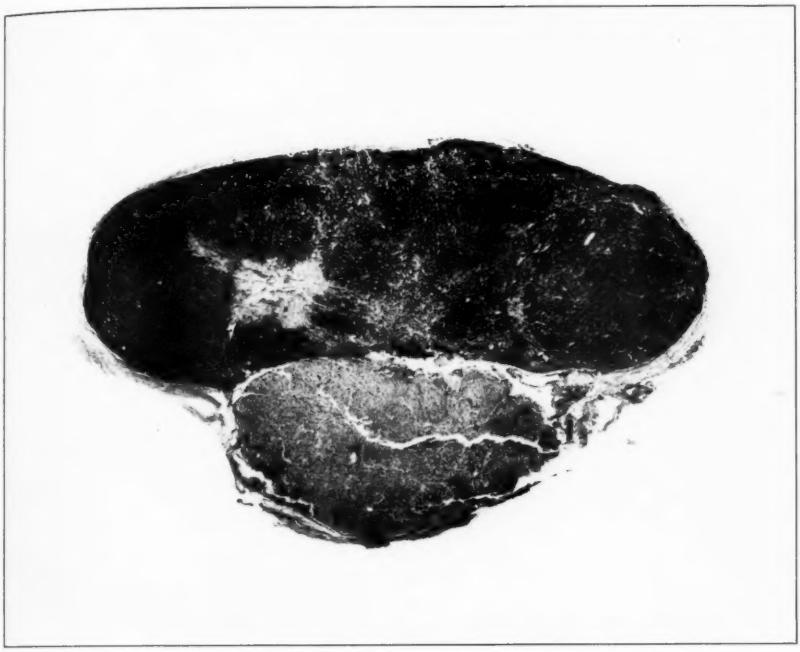
## DESCRIPTION OF PLATES

### PLATE 32

FIG. 1. Photomicrograph of horizontal section of pituitary body from a male of 85 years with blood pressure of 135 systolic and 80 diastolic. Death from uremia, hydronephrosis and overgrowth of prostate. Note absence (−) of basophil cells in neurohypophysis. Hematoxylin-eosin stain. × 8.

FIG. 2. Pituitary body from a negress of 45 years with blood pressure of 205 systolic and 130 diastolic. Death from syphilitic mesaortitis and meningitis. Arrow indicates slight (+) basophil collection in neurohypophysis. Hematoxylin-eosin stain. × 8.



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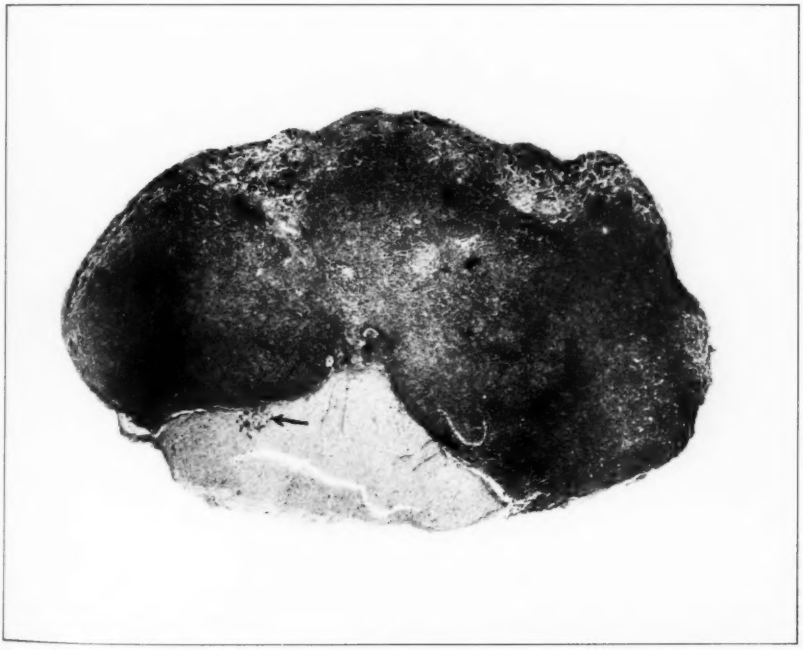


2

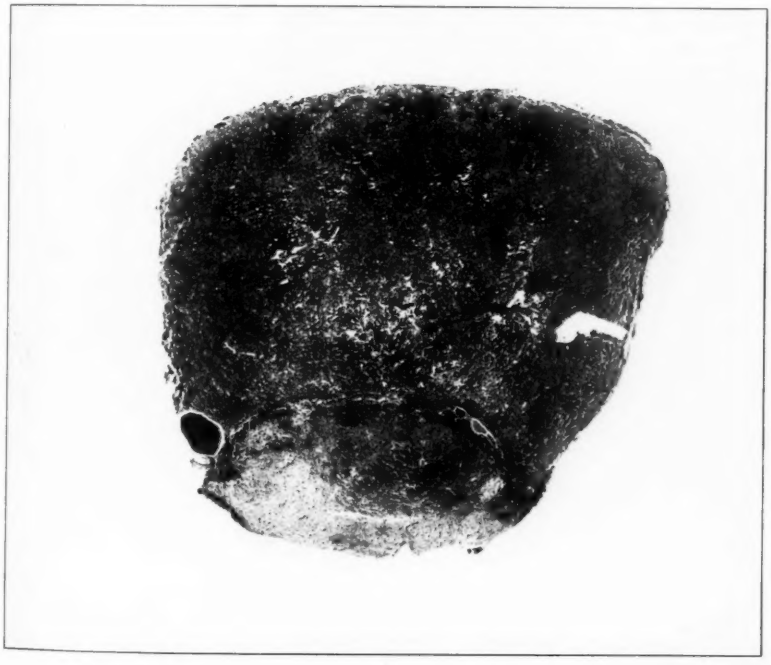
Leary and Zimmerman Basophil Infiltration in Neurohypophysis

PLATE 33

FIG. 3. Photomicrograph of horizontal section of pituitary body from a male of 40 years of age with blood pressure of 190 systolic and 140 diastolic. Death from ruptured dissecting aortic aneurysm. Note moderate (++) basophilia in neurohypophysis below cleft in pars intermedia. Hematoxylineosin stain. × 12.

FIG. 4. Photomicrograph of horizontal section of pituitary body from a male of 57 years with blood pressure that varied between 170 and 250 systolic and 90 and 130 diastolic. Death from arteriosclerotic heart disease with congestive heart failure. Note marked (+++) basophil infiltration in neurohypophysis. Rasmussen's modification of Mallory's connective tissue stain. × 8.

3



4

Leary and Zimmerman Basophil Infiltration in Neurohypophysis

# LOCAL ANAPHYLACTIC LESIONS OF THE BRAIN IN GUINEA PIGS*

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Since Arthus and Breton [1] in 1903 described the histology of the Arthus phenomenon in the rabbit's skin, a great deal of work has been done on the histology of local anaphylactic inflammation in various organs and tissues. Little, however, has been done on the finer histopathology of such inflammation within the central nervous system. Besredka [2] first employed the central nervous system as a route of administration of antigen in his experiments on general anaphylaxis, giving his intoxicating injections subdurally or intracerebrally; but he makes no mention of local changes at the site of injection. Rachmanow [3] and several subsequent workers have described changes (in some cases equivocal in our opinion) in the brains of animals following general anaphylaxis. Experimental allergic or local anaphylactic meningitides have been produced by Burn and Finley [4] with tuberculin, and by Ssolowjew and Ariel [5] with horse serum. But apart from recent reports by Davidoff, Seegal and Seegal,[6] and Davidoff, Kopeloff and Kopeloff, [7, 8, 9] we have been unable to find any studies of local anaphylactic inflammation within brain tissue.

In most of their reports these latter authors focused their interest mainly on clinical phenomena. In one of their papers, however, Davidoff, Seegal and Seegal [6] presented histological data. In rabbits prepared by multiple sensitizing injections they found an extensive inflammatory lesion in the brain following intracerebral reinjection, characterized by hemorrhage, edema, serous exudate and leukocytic infiltration. In distinct contrast to the changes in the brains of the sensitized animals was the paucity of abnormal findings in the brains of the controls in their study. With few exceptions the control brains failed to give any indication of the location of the

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injection. In only two of the brains from control animals was a little free blood found along what was obviously the needle track. This hemorrhage was very slight, was surrounded by normal, non-edematous brain tissue, and showed no leukocytic or other infiltration.

The purpose of our study was to confirm these observations and to attempt to analyze the pathological mechanisms concerned in the production of the lesions, approaching the problem by the more clear-cut method of a single sensitizing injection and a single reinjection, such as is known adequately to produce a local anaphylactic reaction in the guinea pig.

## Material and Methods

Our work is based on the study of the brains and other organs of 39 guinea pigs, 18 of which were sensitized and 21 of which were used as controls. Horse serum exclusively was used for the antigen. The sensitized guinea pigs received as their sensitizing injection a single dose of 0.5 cc. horse serum intraperitoneally. The interval between sensitizing and reinjection was 21 days in all animals of Series A, and those of Series B, except for 3 guinea pigs in which the interval was 14 days. The reinjection consisted of 0.1 cc. horse serum injected into one cerebral hemisphere. For the reinjection the animals were anesthetized with ether and the brain exposed by a small trephine hole under aseptic conditions. A fine needle (25 gauge) was pushed into the hemisphere with as little lateral movement as possible, and the serum injected slowly; in this way mechanical trauma was reduced to a minimum. The control animals received a single intracerebral injection administered in the same way.

The animals were killed by chloroform inhalation at an interval after the intracerebral injection varying from 24 hours to 7 days. Only 1 sensitized animal died spontaneously — in less than 24 hours. In Series A the brains and other ograns (liver, spleen and lungs) after removal were fixed in a mixture of absolute alcohol 9 parts, and pure liquor formaldehyde 1 part. The brains were sliced into blocks and then sectioned. Sections showing the lesion were cut serially. For staining, hematoxylin-eosin, Nissl's and Masson's trichrome methods, and in some cases Heidenhain's iron hematoxylin, were used. Neighboring sections of all the stained sections, 4 $\mu$ thick, were incinerated according to Scott's [10] modification of Poli-

card's [11] method.* The brains of the guinea pigs in Series B were studied by methods adapted to demonstrate the vascular tree; in 14 animals, 7 of which were sensitized and 7 of which were controls, thick frozen sections 200 $\mu$ thick were stained by the Lepehne [12] and Pickworth [13] technique, which demonstrates the vascular tree by staining selectively the contained red blood cells. In 1 sensitized and 1 control animal an India ink injection through the heart was made at the time of killing. A small series of rabbits was also studied with, however, some variation in technique, details of which are given later in the text.

In all instances the lesions were measured in the sections and the maximum transverse diameter recorded. The total length of the lesions was ignored as it depended largely on the depth to which the needle had been pushed in at the time of injection. The comparative size of the lesions in sensitized and control animals can be seen in Table I.

## Protocols †

### *Sensitized Guinea Pigs*

*Guinea Pig A 1:* Reinjected into the right cerebrum. Three hours after injection violent convulsions with rapid tonic-clonic movements of all limbs occurred. The head was rotated and turned to the left during seizures. Convulsions were increased by passive movements. Twenty-two hours later the convulsions ceased, but there was persistent rotation of the head to the left. The gait was jerky and slow. The animal was killed at 30 hours.

*Autopsy:* The brain shows the needle track passing between the occipital lobes, through the midbrain and widening out into the lesion in the lateroventral part of the left side of the pons.

*Histology:* The lesion in the pons measures 3360 $\mu$ in diameter. It consists of an area of extensive hemorrhage and necrosis affecting the gray and white matter (Fig. 1). The area of necrosis shows a loss of ganglion cells, diffuse loosening of the glial reticulum and a scattered neutrophilic polymorphonuclear infiltration (Fig. 8). In certain areas the polymorphonuclears occur in dense groups. There is some perivascular infiltration. No eosinophilic leukocytes are present. Throughout the necrotic area are many thrombosed capillaries and precapillaries which are dilated and filled with deeply staining, homogeneous thrombi (Fig. 3).

Microincinerated preparations show gross demineralization of the necrotic area (Fig. 2). The mineral ashes in the hemorrhage appear as granular white

* Paraffin sections cut at 4 $\mu$ thickness are exposed to a temperature rising to 650° C. Thereby all organic components of the tissue are burnt out and only the mineral ash remains on the slide, in the original topographical arrangement. When studied with darkfield illumination or with oblique transillumination the minerals appear light against a dark background.

† Full protocols of only some of the guinea pigs are given. The main data on all animals are summarized in Table I.

TABLE I

*Series A. Clinical Symptoms, Location, Age, Size and Individual Components of Cerebral Lesions Produced by Injections of 0.1 cc. of Sterile Horse Serum Into the Brains of Sensitized and Non-sensitized Guinea Pigs*

*Sensitized Guinea Pigs*

| Guinea pig No. | Location of lesion | Age of lesion | Size of lesion | Hemorrhage | Thromboses | Necrosis | Infiltration: Polymorphonuclears | Infiltration: Eosinophils | Scavenger cells | Glial reaction: Microglial and oligodendroglial | Glial reaction: Astrocytes | Degree of demineralization | Clinical phenomena |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 13 | Striatum, thalamus, ventricle | Less than 24 hrs. (died) | μ 1170 | +++ | ± | ± | ++ | + | − | − | − | + | Convulsions. Death in less than 24 hours |
| 1 | Midbrain, pons | 30 hrs. | 3360 | +++ | ++ | +++ | ++ | − | − | − | − | +++ | Prolonged convulsions |
| 2 | Thalamus, midbrain | 30 " | 3300 | +++ | ++ | +++ | ++ | − | − | ± | − | +++ | Prolonged convulsions |
| 3 | Midbrain, tegmentum pontis | 30 " | 3230 | +++ | ++ | +++ | ++ | ± | ± | ± | − | +++ | Prolonged convulsions |
| 7 | Temporal lobe | 2 days | 2500 | +++ | ++ | +++ | ++ | − | ± | − | − | +++ | Diminution of activity. Ruffled hair |
| 14 | Parietal lobe, postcentral region | 4 " | 420 | + | + | + | ± | − | + | + | ± | + | Brief convulsions |
| 15 | Parietal lobe | 4 " | 2100 | ++ | ++ | +++ | ++ | − | +++ | + | ± | +++ | Brief convulsions |
| 19 | Postcentral region, capsula externa | 7 " | 400 | ++ | − | − | − | − | ± | + | ± | None. Iron in scavengers | |
| 20 | Postcentral region | 7 " | 280 | + | − | − | − | − | ± | + | + | None. Hypermineralization of gliosis | |
| 21 | Parietal lobe | 7 " | 270 / 17,030 (Average 1703) | − | ± | − | − | − | − | + | ± | None. Hypermineralization of gliosis | |

*Non-sensitized Guinea Pigs*

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | lobe | | 17,030<br>(Average 1703) | | | | | | | | | None. Hypermineralization of gliosis | |

*Non-sensitized Guinea Pigs*

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 4 | Midbrain | 30 hrs. | 540 | + | + | + | + | − | − | − | − | + | |
| 5 | Midbrain | 30 " | 90 | + | − | − | + | − | − | ± | − | ± | |
| 6 | Thalamus | 30 " | 630 | + | ± | ± | + | − | − | − | − | ± | |
| 10 | Cornu ammonis | 2 days | 430 | + | ± | ± | + | − | ± | − | − | ± | |
| 11 | Temporal lobe | 2 " | 350 | + | + | ± | + | − | − | ± | − | ± | |
| 25 | Midbrain | 2 " | 300 | + | − | − | ± | − | − | + | ± | None | Brief convulsions. Transient ruffling of hair |
| 26 | Temporal lobe | 2 " | 400 | + | − | − | + | − | − | ± | − | None | |
| 16 | Parietal lobe | 4 " | 640 | + | ± | + | − | − | ++ | + | + | + | |
| 17 | Parietal lobe | 4 " | 240 | − | − | − | − | − | − | + | − | None | |
| 18 | Striatum | 4 " | 370 | + | − | − | − | − | ± | + | ± | ± | Transient convulsions |
| 22 | Parietal lobe | 7 " | 320 | − | ± | − | − | − | ± | + | ± | None. Hypermineralization of gliosis | |
| 23 | Postcentral region, capsula extrema | 7 " | 380 | + | − | + | − | − | ++ | + | ± | None. Hypermineralization of gliosis | |
| 24 | Parietal lobe | 7 " | 290<br>4980<br>(Average 383) | − | − | − | − | − | ± | + | ± | None. Hypermineralization of gliosis | |

− = absent
± = present in slight degree
+ = present in moderate degree
++ = present in greater degree
+++ = present in advanced degree

and yellow dots on a bluish background. The meninges show a slight patchy infiltration with equal numbers of neutrophilic polymorphonuclears and mononuclears.

*Guinea Pig A 2:* Reinjected into the right cerebrum. Convulsions were noted after 2 hours and persisted up to 30 hours when the animal was killed. The convulsions occurred in rapid succession as in status epilepticus. The animal was unresponsive to stimuli, except for an increased outburst of jactational convulsions when moved. Two types of convulsion were observed: (1) jactational convulsions around the sagittal axis; and (2) tonic-clonic convulsions of all limbs, increased on the right, with quick flexor (1/16 second) and slower extensor movements (1/4 second). During these convulsions the animal lay on its left side with its head turned and rotated to the left.

*Autopsy:* The brain shows the needle track entering the right parietal lobe, extending through the right thalamus and widening into a large lesion in the right half of the midbrain, destroying the right tegmentum, including the lateral part of the red nucleus, and the right peduncle (Figs. 4 and 5), and extending backward into the right pons.

*Histology:* The lesion measures 3300 $\mu$ in diameter. It consists of hemorrhage and necrosis with neutrophilic polymorphonuclear infiltration. The lesion is similar to that in Guinea pig A 1, except that there is a slight proliferation of pale glial nuclei (mainly microglia but with slight increase of oligodendroglia) around the hemorrhage. In the microincinerated preparations the demineralized area is more clear-cut, giving a plaque-like appearance (Fig. 5). The hemorrhage appears richer in iron granules.

*Guinea Pig A 3:* Reinjected into the right cerebrum. After 1½ hours frequent convulsions developed, tonic-clonic in type, affecting all limbs but mainly the left. The head turned to the left but rotated to the right. These convulsions lasted 3 hours. Later the animal appeared normal, apart from a leftward tendency in gait. It was killed at 30 hours.

*Autopsy:* The needle track pierced the tip of the right occipital lobe, went through the posterior corpora quadrigemina, widened into a wide hemorrhagic lesion in the right tegmentum of the pons, extended to the floor of the fourth ventricle, and included vestibular and trigeminal nuclei (Figs. 6 and 7).

*Histology:* The lesion measures 3230$\mu$ in diameter. It is essentially similar to those of Guinea pigs A 1 and A 2, showing ischemic necrosis, especially of ganglion cells (Fig. 9), hemorrhage, thromboses and infiltration with neutrophilic polymorphonuclears. A notable feature is the sharply outlined area of necrosis and demineralization (Figs. 6 and 7). The central part of the lesion, which in the hematoxylin and eosin, iron hematoxylin, and Masson stained sections fails to show complete necrosis (Fig. 6), nevertheless is seen in microincinerated preparations to be almost fully demineralized (Fig. 7). This suggests that loss of mineral is an early change in the development of this type of necrosis. While most of the thrombi consist of homogeneous masses plugging capillaries and precapillaries, one large venule is occluded by a thrombus that shows a distinct fibrinous network containing red cells and leukocytes in its meshes. Fibrinous strands are adherent to the necrotic wall of the vessel. In some of the veins in the lesion eosinophils are fairly numerous, but only an occasional one is seen extravascularly. Occasional scavenger cells are seen also in the necrotic area. The hemorrhage in the microincinerated preparations shows very clearly a moderate amount of iron oxide granules.

*Guinea Pig A 13:* Reinjected into the right cerebrum. After 15 minutes violent tonic-clonic convulsions of all four limbs occurred, lasting 15 to 20 minutes. Later the head and the trunk were noted to be flexed to the left. The animal was slightly ataxic and showed scratching at the nose, suggestive of a mild general anaphylaxis. Later it improved somewhat, but died unexpectedly during the night.

*Autopsy:* An extensive hemorrhagic lesion is present in the right striatum, central white matter, corpus callosum, and adjacent parts of the right and left cornu ammonis. An extension spreads to the left, affecting the ventromedial part of the left thalamus and the left internal capsule. There is also an extensive ventricular hemorrhage, presumably the cause of death.

*Histology:* The lesion measures 1170 $\mu$ in maximum diameter, and consists of a large and a few small satellite perivascular hemorrhages, increased polymorphonuclear infiltration, especially around these areas but also around vessels which show no hemorrhage. These vessels are dilated and filled with blood, but only a few of them present thrombi. In this lesion the thrombi are somewhat smaller and less homogeneous than those seen in Guinea pigs A 1 and A 3. They consist of closely packed masses of red cells intermingled with polymorphonuclears and a few strands of fibrin, most notable where the clots adhere to the vascular wall. Most of the vessels show only congestion, probably to be interpreted as a prethrombotic stasis. The ganglion cells surrounding the hemorrhages show slight pyknosis, but there is no necrosis of the tissue as a whole and no glial reaction. Demineralization is seen around the infiltrated vessels and the hemorrhages, but there is no massive demineralization as in Guinea pigs A 1, A 2, and A 3. The lesion shown is earlier than 24 hours. The absence of massive demineralization probably corresponds to the paucity of fully developed thromboses. Eosinophils are seen in fair numbers around some of the infiltrated vessels but do not extend out into the more diffuse infiltration.

*Guinea Pig A 14:* Reinjected into the right cerebrum. After 15 minutes a single brief clonic convulsion of both rear limbs was noted. Thereafter no abnormality, apart from scratching at the nose, suggestive of a mild general anaphylaxis, was seen. The animal was killed at 4 days.

*Autopsy:* The needle track is in the right parietal lobe. No gross lesion can be seen macroscopically.

*Histology:* The needle track ends in a small lesion in the subcortical white matter of the right postcentral region. The lesion is 420 $\mu$ in its maximum diameter and consists of an area of hemorrhage surrounded by a narrow zone of necrosis. A few scavenger cells are present at the periphery of the hemorrhage. The zone of necrosis shows proliferation of microglia and oligodendroglia, with a few astrocytes, and an occasional polymorphonuclear leukocyte. A few thromboses of capillaries and one large venule are seen. This lesion resembles those seen in the control animals, rather than those seen in the definitely anaphylactic pigs. It seems probable that sensitization was not successful in this animal.

*Guinea Pig A 15:* Reinjected into the right cerebrum. Fifteen minutes later a few, brief, general convulsions were noted. Thereafter no abnormal clinical change was seen. The animal was killed at 4 days.

*Autopsy:* The needle track enters the right parietal lobe and widens into a gross lesion extending from the fifth layer of the cortex to the lateral ventricle (Figs. 10 and 11).

*Histology:* A large lesion 2100 $\mu$ in maximum diameter is present consisting of a hemorrhage of moderate size in the subcortical white matter and a much larger area of softening with many Gitter cells (Fig. 12). An increased neutrophilic polymorphonuclear infiltration is present around the vessels both at this point and in the normal surrounding tissue (Fig. 12). There is a slight proliferation of oligodendroglial and microglial tissue around the area of softening. A few astrocytes are also seen. The blood vessels are greatly dilated and capillaries are somewhat increased in number. A few of the vessels show thrombi. An occasional intravascular eosinophil is seen, no one of which is infiltrating. In microincinerated preparations the lesion appears grossly demineralized (Fig. 11). On the demineralized background, however, the scavenger (Gitter) cells and the nuclei of infiltrating polymorphonuclear leukocytes stand out intensely mineralized. The Gitter cells contain mineral in their nuclei and cytoplasm (Fig. 13). Some of the granular cytoplasmic mineral deposit is iron oxide, but it is mainly a white, water insoluble ash.

*Guinea Pig A 19:* Reinjected into the right cerebrum. No clinical abnormalities followed. The animal was killed at 7 days.

*Autopsy:* The needle track enters right postcentral region and ends in a narrow hemorrhagic streak in the external capsule.

*Histology:* A sheet-like, circumscribed lesion measuring 400 $\mu$ in maximum diameter is seen occupying the external capsule. It consists of hemorrhage with a slight surrounding glial proliferation. There is no evidence of glial fiber formation. No polymorphonuclear leukocytes are seen and there are no thromboses. Microincineration shows no demineralization. Fairly plentiful iron is seen along the edges of the hemorrhage, most of it concentrated in scavenger cells lining the hemorrhagic cavity. It is also found around smaller vessels in the neighborhood of the lesion. These same scavenger cells are seen in hematoxylin and eosin preparations as cells with fine brown cytoplasmic granules.

*Guinea Pig A 21:* Reinjected into the right cerebrum. No clinical manifestations were present. The animal was killed at 7 days.

*Autopsy:* The needle puncture is in the right parietal lobe. No macroscopic lesion can be seen on sectioning the brain.

*Histology:* A narrow glial scar is seen running from the surface of the parietal lobe through the cortex and subcortical white matter into the corona radiata. It consists of proliferated microglia and oligodendroglial cells with occasional astrocytes (Fig. 15). The ganglion cells have disappeared within the scar and the surrounding tissue appears unaffected. A thrombosed precapillary vessel (Fig. 14) with dilated capillary branches is also seen in the scar. A few fibroblasts and endothelial buds are seen in relation to these dilated capillaries. The lesion measures 270 $\mu$ across its widest area. Microincinerated preparations show hypermineralization in the area of the scar. Granules of iron are seen in some of the microglia cells. The surrounding tissue shows a normal mineral picture.

*Non-sensitized Guinea Pigs*

*Guinea Pig A 4:* Single injection into the right cerebrum. No clinical manifestations were noted. The animal was killed at 30 hours.

*Autopsy:* The needle track traverses the right occipital lobe and enters the right half of the midbrain. No macroscopic necrosis is noted.

*Histology:* The maximum diameter of the lesion is 540 μ. The lesion consists of a narrow streak of hemorrhage with a few tiny foci of necrosis in the immediate periphery (Fig. 16), which in microincinerated preparations show demineralization (Fig. 17). There is a scanty neutrophilic polymorphonuclear infiltration of these areas (Fig. 18). No eosinophils are present. A few thrombosed capillaries are seen immediately surrounding the hemorrhage. The meninges overlying the lesion show considerable localized infiltration with neutrophils.

*Guinea Pig A 10:* Single injection into the right cerebrum. No clinical manifestations were noted. The animal was killed at 48 hours.

*Autopsy:* A small hemorrhagic streak is seen in the cornu ammonis of the right hemisphere. No necrosis can be seen macroscopically.

*Histology:* The lesion measures 430 μ in diameter and consists of an area of hemorrhage surrounded by a narrow zone of necrosis in which a few thrombosed capillaries are seen. There is a moderate, diffuse, neutrophilic polymorphonuclear infiltration immediately surrounding the hemorrhage and a few scavenger cells are seen at the periphery. No eosinophils are present and there is no glial reaction.

*Guinea Pig A 25:* Single injection into the right cerebrum. After 15 minutes there was a short series of tonic-clonic convulsions lasting 10 minutes and affecting all limbs, especially the right. There was also a transient ruffling of the hair. The animal was killed at 48 hours.

*Autopsy:* The needle track traverses the right occipital lobe and enters the right midbrain. No macroscopic necrosis is visible.

*Histology:* A circumscribed area of hemorrhage surrounded by a narrow rim (2 rows deep) of early oligodendroglial and microglial proliferation with an occasional polymorphonuclear leukocyte is seen. There is a slight astrocytic proliferation outside the oligodendroglial and microglial rim. No thromboses are seen.

Microincineration shows no demineralization. Granules of iron oxide are present in the hemorrhage. The maximum diameter of the lesion is 300 μ.

*Guinea Pig A 16:* Single injection into the right cerebrum. No clinical manifestations were present. The animal was killed at 4 days.

*Autopsy:* There is a pin-point needle puncture in the right parietal lobe, but no macroscopic evidence of necrosis.

*Histology:* The lesion measures 640 μ in maximum diameter. It consists of a small hemorrhagic cyst in the subcortical white matter (Figs. 19 and 20) containing many Gitter cells (Fig. 21). Surrounding it is a small, localized zone of necrosis with dense glial proliferation of microglial and oligodendroglia cells and astrocytes (Fig. 21). No polymorphonuclear leukocytes and no eosinophils are present. Neighboring vessels show slight dilatation. One at the periphery of the hemorrhage shows thrombosis.

Microincineration shows a small zone of slight demineralization surrounding the hemorrhage (Fig. 20), which contains granules of iron oxide.

*Guinea Pig A 22:* Single injection into the right cerebrum. No clinical manifestations were observed. The animal was killed at 7 days.

*Autopsy:* No lesion is found macroscopically, apart from the localized adherence of the dura on the right parietal lobe under the trephine hole.

*Histology:* A small area of gliosis showing oligodendroglia and a few astrocytes, 320 $\mu$ in maximum diameter, is seen in the subcortical white matter.

Microincinerated preparations show hypermineralization of this glial focus, and a few iron-containing scavenger cells are seen in the perivascular spaces around two small vessels.

*Guinea Pig A 23:* Single injection into the right cerebrum. No clinical manifestations were noted. The animal was killed at 7 days.

*Autopsy:* The needle track enters the right postcentral region and extends into the central white matter and the capsula extrema. No macroscopic signs of necrosis are visible.

*Histology:* A circumscribed lesion with a maximum diameter of 380 $\mu$ is present. There is a hemorrhagic cyst, the capsule of which consists of a double rim. The inner rim is made up of microglia and oligodendroglia; the outer rim of proliferating astrocytes. It is thin and sharply marked off; the nerve cells inside the rim show severe changes, but on the outside they are well preserved. There is no demineralization or evidence of necrosis outside the glial rim. The hemorrhagic cyst contains many large Gitter cells, as well as numerous red blood cells.

Microincinerated preparations show iron oxide granules, some free in the hemorrhage, others in scavenger cells in the hemorrhage and at the margin. The inner microglial and oligodendroglial rim shows hypermineralization, both nuclear and cytoplasmic, and it is also seen in the processes of some of these cells. The outer astrocytic rim shows hypermineralized plump cells bodies and processes, the mineral being deposited in a finely granular form.

## Changes in Organs Other Than the Brain

Examination of the other organs showed, in the sensitized guinea pigs, slight fatty changes in the liver, most extreme in the centers of the lobules. The livers of the non-sensitized animals, and the lungs and spleens of both sensitized and non-sensitized groups, showed no significant abnormality.

## Vascular Phenomena in the Lesions

In the guinea pigs of Series B (8 sensitized and 8 control animals) an attempt was made to investigate in particular the vascular phenomena in the intracerebral lesions. Brains examined by the Lepehne-Pickworth method showed in both the sensitized and the control animals an area of hemorrhage surrounded by a zone of anemia (Figs. 22, 23 and 24). The center of the hemorrhage was too dense to permit any vascular detail to be seen; at the periphery, however, there were many individual satellite perivascular hemorrhages, and in some instances vessels in this area showed localized dilatation and bead-like deformities, which probably denote thrombosis of the vessels (Alexander and Putnam [14]). We were in some

doubt as to the successful sensitization of some of the animals in this group, since the size of the total lesions and of the hemorrhages, although greater on the whole in the sensitized animals, was not very significantly so. The anemic zone, however, did appear distinctly wider in most of the sensitized animals (compare Figs. 22 and 23). The explanation of this anemic zone (Fig. 24) is, we feel, probably the expression of the red cells out of the capillaries by edema. It is to be noted that hemorrhage and edema, although greater in the sensitized animals, were also characteristic of the lesions in the control animals.

In the 2 animals injected with India ink the ink did not penetrate vessels in the major hemorrhagic mass and did not extravasate into the hemorrhage. We deduce, therefore, a complete occlusion of the vessels in this area. The zone around the hemorrhage, corresponding to the anemic area in the Lepehne-Pickworth preparations, was, however, perfectly injected.

### Sensitized and Non-sensitized Rabbits

In this series 6 rabbits were used (3 sensitized, 3 controls). In contrast to the guinea pigs, which were sensitized with a single dose, we used in the rabbits a method resembling rather the classical Arthus procedure, *i.e.* sensitization was effected by giving five injections of horse serum, 2 cc. each intraperitoneally, at 5 day intervals. The intracerebral injection of 0.2 cc. horse serum was given after a further interval of 10 days. The 3 controls received only the intracerebral injection. All 6 rabbits were then killed by chloroform after 3 days.

#### *Results*

No clinical abnormalities were noted. At autopsy, in 2 of the sensitized rabbits a large hemorrhagic necrotic lesion was seen macroscopically on slicing the brain. In the other sensitized rabbit a hemorrhagic track was found. In none of the controls could any lesion be found macroscopically. Serial sections were studied.

#### *Histological Examination*

As in the guinea pig series, there was a definite contrast between the size of the lesions in the sensitized and in the control groups. The lesions in the sensitized group measured 1500 and 2500 $\mu$ (Figs. 25 and 26), and 1900 $\mu$ (Fig. 27) respectively. In the controls the

lesions measured 400 μ (Fig. 28) and 320 μ, respectively. No lesion could be found in the 3rd rabbit of the control series. It was probably lost in trimming the paraffin blocks.

The lesions in the sensitized rabbits were comparable in general to those in the sensitized guinea pigs. They showed hemorrhage, necrosis, and scavenger cell and glial infiltration with vascular thrombosis. No polymorphonuclears or eosinophils were seen. The areas of necrosis contrasted with those in the guinea pigs in that they were demineralized only in certain parts and in other areas were highly hypermineralized (Fig. 26). The hypermineralized areas showed in sections stained with hematoxylin and eosin and Masson's trichrome stains (Fig. 25) a striking fibrinous exudation such as was not seen in the guinea pigs. The hypermineralization of the fibrinous exudation exceeded in degree and density the hypermineralization of the hemorrhages.

The lesions in the controls showed mere hemorrhagic streaks (Fig. 28) with Gitter cell and microglial and oligodendroglial reaction with necrosis of ganglion cells in the immediate vicinity. There was no dissolution of the surrounding tissue, no demineralization or fibrinous exudate and no thromboses.

In the 3 sensitized rabbits, apart from the gross localized lesion, occasional lymphocytic perivascular infiltrations were found widespread through the brain, and in 2 of the 3 there were occasional microglial and oligodendroglial foci. While these changes may have been caused by our procedures, in view of the ease of production in rabbits of such an encephalitis by other procedures, and also in view of their occasional spontaneous occurrence, we can attach to them no specific importance.

Examination of other organs of the sensitized rabbits showed changes similar to those observed by Apitz [15] in similarly treated rabbits, namely lymphocytic and histiocytic periportal infiltration in the liver and enlargement of the spleen with increase in number of large mononuclear cells similar to those seen in acute splenic tumor.

## Discussion

From our findings we conclude that local anaphylactic disease can be produced in the brains of guinea pigs. By reinjecting horse serum into the brains of sensitized guinea pigs clinical symptoms and gross

macroscopic lesions can be produced, while single intracerebral injections in the non-sensitized controls cause only rare and transient clinical symptoms and little or no macroscopic damage. The histological examination showed also an appreciable difference between the lesions of the two groups. A striking feature, however (summarized in Table II), is the non-specificity of any individual com-

TABLE II

*Individual Components of Cerebral Lesions Produced by Injection of 0.1 cc. of Sterile Horse Serum into the Brains of Sensitized and Non-sensitized Guinea Pigs. (Compiled From the Maximum Lesions in Table I)*

| Lesion | Sensitized guinea pigs | Non-sensitized guinea pigs |
|---|---|---|
| Hemorrhage | +++ | + |
| Thrombosis | ++ | + |
| Necrosis | +++ | + |
| Neutrophilic polymorphonuclear leukocytes | ++ (4 days) | + (2 days) |
| Eosinophils | + (in 2 animals only) | − |
| Scavenger cells | +++ | ++ |
| Microglia and oligodendroglial cells | + | + |
| Astrocytes | + | + |
| Demineralization | +++ | + |

ponent of the histological picture in the lesions in sensitized and in control animals. Briefly, we see in the sensitized animals at the site of reinjection a lesion consisting of hemorrhage, vascular thromboses,* necrosis, demineralization and infiltration with neutrophilic polymorphonuclear leukocytes, and microglial and oligodendroglia cells. Later scavenger cells and astrocytes proliferate. Although these changes, apart from the glial proliferation, reach their maximum in the group of sensitized animals, none of them is absent from the group of control animals. Sterile horse serum injected into the non-sensitized guinea pig's brain produces a lesion that may show all the features cited above. The hemorrhage, the vascular thromboses, the necrosis and demineralization are, however, vastly more extensive in the sensitized group. The average diameter of the lesion in these animals, (1703 $\mu$) as compared with that of the lesion in the

* The thrombosed capillaries and precapillary blood vessels in our animals were dilated, showed necrosis of the walls, were filled with adherent, deeply staining, homogeneous or amorphous fibrinous material and were impermeable to injection fluid. In only few exceptional instances of thrombi in precapillary vessels were distinct fibrinous strands, accumulation of white cells and beginning organization observed.

controls (383 $\mu$) shows the striking difference in degree and intensity of the pathological process in the two groups.

This finding of a quantitative rather than qualitative difference between the anaphylactic and the non-anaphylactic lesion is in accord with the conclusions of Gerlach.[16] Gerlach, after a comprehensive review of the histology of the hyperergic reaction in several species, states that the characteristic feature of this reaction is the much greater intensity of the process. He denies the existence of any specific qualitative feature, thus differing from the opinion of Rössle[17] who, while stressing also the difference in speed and intensity of the reaction, referred also to an unusual and specific qualitative feature in the form of a high percentage of eosinophilic leukocytes among the infiltrative cells. Since Schlecht and Schwenker [18] in 1912 reported eosinophilic infiltration in local and general anaphylaxis many observers have confirmed its occurrence. It is, however, although apparently a specific feature of local anaphylactic inflammation, by no means a constant one (Campbell, Drennan and Rettie [19]). In the intracerebral lesions in our guinea pigs it was present only to a moderate degree in one animal and only to a minor degree in another. Both these animals were sensitized. In view of its rarity in our series we feel that it does not detract from the general statement that the specific characteristics of the local anaphylactic lesions are quantitative rather than qualitative.

As to the pathogenesis of the lesions in our animals, the two alternative possible mechanisms of production of these necrotic lesions seem to us to be on the one hand primary vascular disturbance, and on the other direct damage to the tissue by the antigen or the antigen-antibody reaction, such vascular lesions as we have described being a secondary or independent accompaniment of the tissue necrosis. We were, however, much impressed by the close resemblance of our lesions to lesions of known vascular origin. The hemorrhages, the thromboses, the more or less sharply outlined areas of necrosis (Putnam [20]) associated with demineralization (Alexander and Myerson [21]) and the appearance of scavenger cells, many of which were loaded with iron granules, all carried out this impression. Furthermore, the larger size of the necrotic hemorrhagic lesion and the greater extent and completeness of demineralization in the sensitized animals could be correlated with a greater number of thromboses in the lesions seen in these animals. Therefore, a

pathogenetic mechanism acting by way of disturbance of the vascular bed with resultant ischemic necrosis seems to us at any rate an important factor.

Such a factor, namely vascular disturbance in anaphylaxis, local or general, has much to support it in the literature. The perfusion experiments on isolated blood vessel preparations of Friedberger and Seidenberg,[22] and of Genes and Dinerstein,[23] showed that when the specific antigen was added to the perfusion fluid the rate of perfusion through sensitized organs was greatly decreased. Fröhlich [24] demonstrated by vital experiment on the mesentery of the sensitized frog that application of the specific antigen produced stasis with capillary dilatation and edema. Dietrich and Nordmann [25] showed by observations on the living mesentery of rabbits that in the peritonitis produced by *B. coli*, if the animals had been previously sensitized by *B. coli* vaccine, the arteries of the inflamed mesentery showed a persistent contraction, in contrast to dilatation of the arteries in unsensitized controls. There is evidence, therefore, of an interference, functional or structural, with the circulation through tissues subjected to anaphylactic phenomena. Histological evidence of definite structural interference is also forthcoming. Dietrich and Schröder [26] demonstrated the increased tendency to thrombosis in the veins of sensitized animals when the specific antigen was injected intravenously. The thrombi consisted of homogeneous fibrinous lamellae and nodules immediately overlying the endothelium, which served as foci of accumulation of platelets, white blood cells and red cells, forming a mixed thrombus. They attributed this process to a sensitization of the endothelium, leading to an intravascular (especially endothelial) Arthus phenomenon. Blood vessels appear to be a tissue readily sensitized and liable to anaphylactic inflammation (Metz,[27] Apitz [15]).

Studies of local anaphylactic lesions in other tissues and organs by several observers have suggested the importance of vascular disturbance, especially occlusion, in the pathogenesis of the lesion. Gerlach [16] in his study on the cutaneous hyperergic reaction emphasizes the appearance of stasis and edema within 1 hour after the anaphylactic injection, followed later by swelling of the connective tissue fibrils to such an extent as to occlude the capillaries and to produce eventually ischemic necrosis. A similar occlusion of capillaries, probably by edema, explains in our opinion the anemic

zone found surrounding the main lesion in those of our guinea pigs examined by the Lepehne-Pickworth method. Opie [28] emphasized the importance of the capillary thromboses which he found in local anaphylactic lesions of the skin of rabbits. He thought that these capillary thromboses were the result of damage to the lining endothelial cells by the occurrence of an antigen-antibody reaction within these cells, and that the capillary thromboses led to ischemic necrosis in the center of the lesion. Klinge [29] noted in the acute anaphylactic inflammation which he produced in joints peculiar subendothelial foci of necrosis which lay in direct relation to small vessels that were usually thrombosed. He did not, however, think that the necroses were ischemic in origin. Long and Finner [30] produced lesions in the kidney, mainly of the nature of a glomerulonephritis, by reinjection of tuberculin into the renal artery in pigs; another group of lesions, however, appeared to be definitely secondary to vascular injury, since subcapsular infarcts in combination with thrombosed venules were found in some instances. Ssolowjew and Ariel [5] produced local anaphylactic (or hyperergic) leptomeningitis in rabbits by injecting horse serum into the cisterna magna; vascular thromboses were a prominent feature in the inflamed meninges, together with necrosis and inflammation of the vessel walls, and were associated with and presumably the cause of focal hemorrhages in the underlying brain substance.

Another matter of interest is the time required for the cerebral lesions and their individual components to develop. Scavenger cells, although isolated examples could be observed after 30 hours in the lesions of sensitized animals, did not appear in any significant number before the 4th day. Polymorphonuclear leukocytes made their appearance on the 1st day and remained in the tissues up to the 4th day in sensitized animals, and up to the 2nd day in those that were non-sensitized. It is interesting to note the difference in the time of appearance of these phenomena between our series of animals and those reported by Alexander, Jung and Lyman,[31] in which intracerebral lesions were produced by a different method, *i.e.* by injection of a colloidal solution.

Thromboses, hemorrhage, necroses and demineralization also appeared within the first 30 hours. Especially interesting is this early appearance of extensive, more or less sharply outlined areas of complete demineralization. The rapid development of demineralization

in foci of ischemic necrosis and softening in humans has been described by Alexander and Myerson [21] in a study of normal and pathological brain tissue by the microincineration technique. The exact mechanism of this rapid demineralization remains obscure. Intracellular iron in scavenger cells derived from ingested red cells was found in microincinerated preparations within the first 30 hours and appeared in considerable quantity at 4 days.

The production of such local anaphylactic lesions in the brains of guinea pigs experimentally has of course no direct counterpart in human pathology. It may, however, have some bearing on the pathogenesis of certain encephalitides, especially those associated with infectious disease, and vaccination for which an anaphylactic factor has been postulated (Glanzmann,[32] Paul,[33] and van Bogaert[34]); and possibly on the rapid progression of others — for instance dementia paralytica. Suggestive of similarity are the speed and intensity of the reaction and the apparent relation of the lesions to vascular disturbances. This point is of interest especially in view of the recent suggestion (Putnam,[35] and Putnam, Merritt and Campbell [36]) of an underlying circulatory disturbance in encephalomyelitis, multiple sclerosis and dementia paralytica.

## Summary

1. Local anaphylactic lesions were produced in the brains of guinea pigs sensitized by a single injection of horse serum intraperitoneally and by reinjection of the antigen intracerebrally.

2. The clinical symptoms produced by these lesions in sensitized animals were prolonged or brief convulsions, diminution of activity, ruffling of the hair and scratching at the nose. Brief convulsions and transient ruffling of the hair were occasionally noted also in non-sensitized animals.

3. The anaphylactic lesions were characterized by hemorrhage, vascular thrombosis, necrosis, demineralization, infiltration with neutrophilic polymorphonuclear leukocytes, scavenger cells, microglial and oligodendroglial cells and astrocytes. Anemia, attributed to compression of capillaries by edema, was observed around the lesions.

4. Similar but much smaller lesions were produced in unsensitized guinea pigs by a single intracerebral injection of antigen.

5. The lesions in the sensitized animals differed quantitatively rather than qualitatively from those in the controls.

6. The pathogenic mechanism of production of the lesions and their possible relation to human pathology are discussed.

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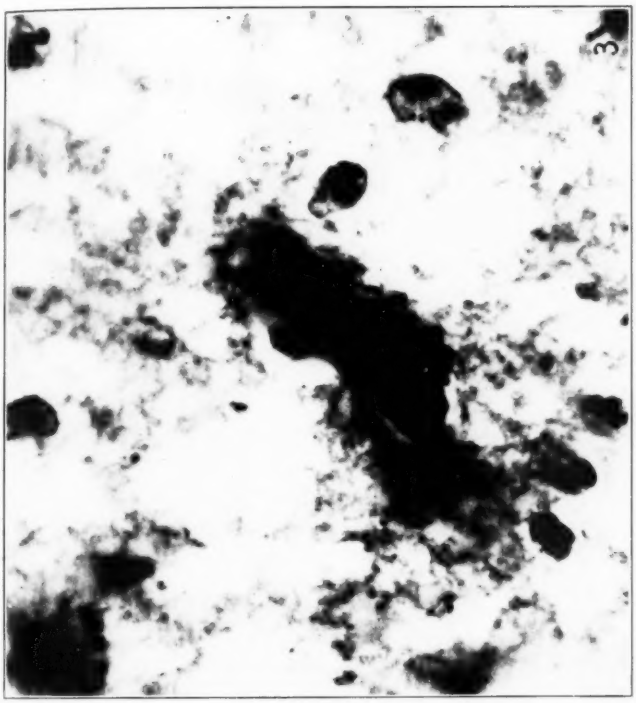
## DESCRIPTION OF PLATES

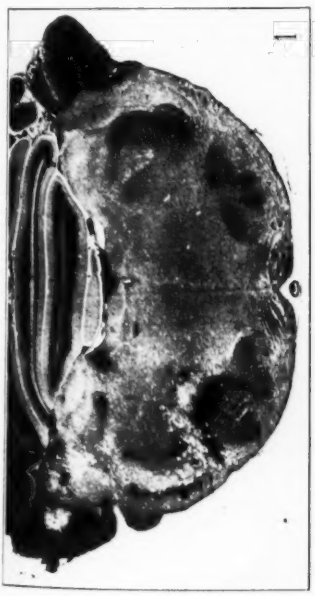
### PLATE 34

FIG. 1. Guinea pig A 1. Sensitized, 30 hour lesion. Masson's trichrome stain. Note hemorrhage and necrosis in the left side of the pons. × 8.

FIG. 2. Guinea pig A 1. Neighboring section to that seen in Fig. 1. Microincineration, oblique transillumination. Note demineralization of necrotic area in the left side of the pons. × 8.

FIG. 3. Guinea pig A 1. Note thrombosed capillary in necrotic area. Masson's trichrome stain. × 1960.





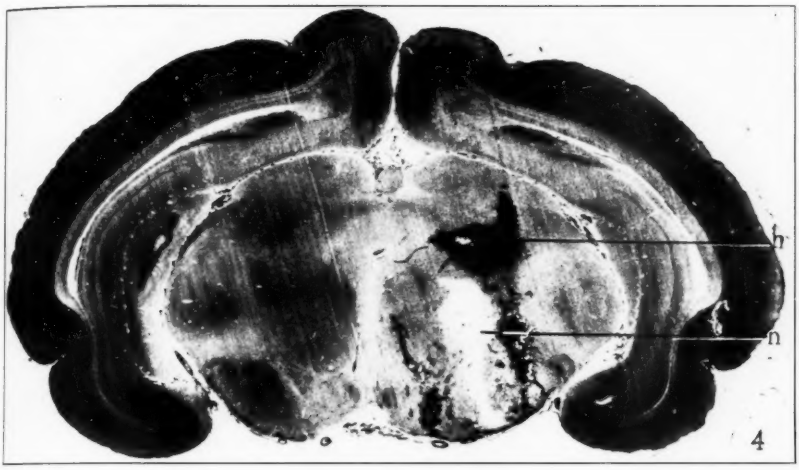
Alexander and Campbell

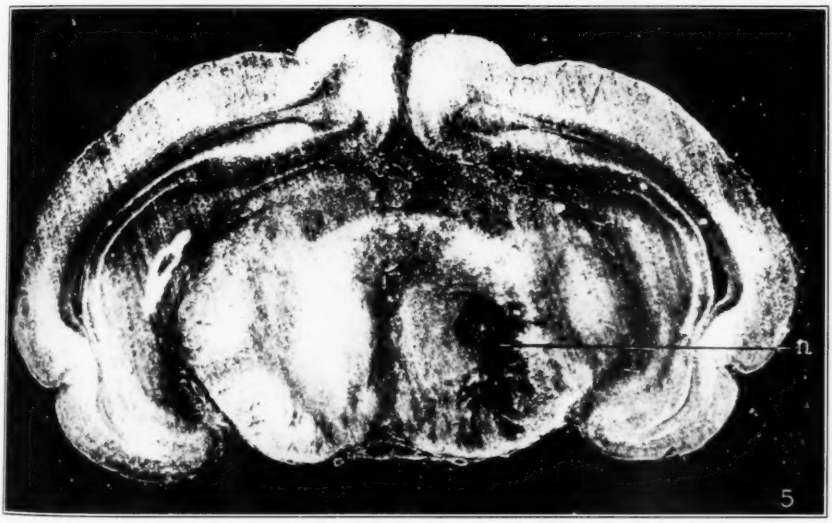
Anaphylactic Lesions in Brain

PLATE 35

FIG. 4. Guinea pig A 2. Sensitized, 30 hour lesion. Note hemorrhage (h) and sharply outlined area of necrosis (n) in the right midbrain. Masson's trichrome stain. × 6.6.

FIG. 5. Guinea pig A 2. Neighboring section to that seen in Fig. 5. Microincineration, oblique transillumination. Note sharply outlined demineralization of necrotic area (n). × 6.6.




Alexander and Campbell Anaphylactic Lesions in Brain

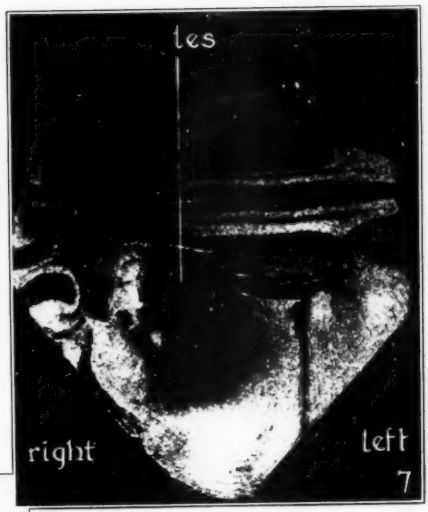
## PLATE 36

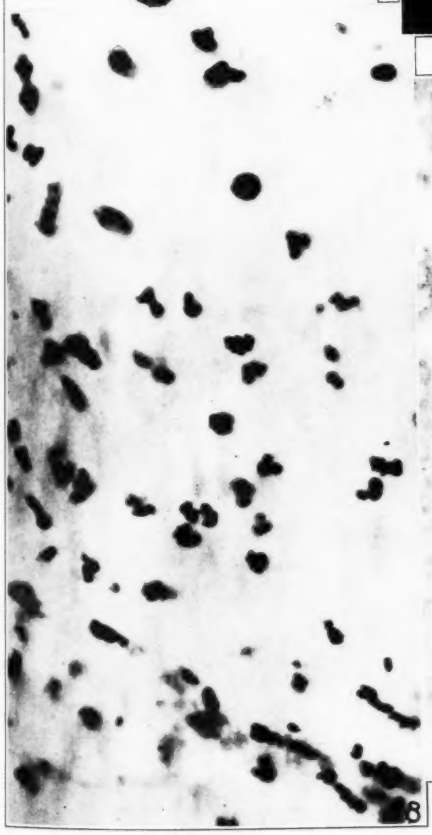
FIG. 6. Guinea pig A 3. Sensitized, 30 hour lesion. Iron hematoxylin. Note hemorrhage and necrosis (les) in the right tegmentum of the pons. Heidenhain's iron hematoxylin stain. × 8.

FIG. 7. Guinea pig A 3. Neighboring section to that seen in Fig. 7. Microincineration, oblique transillumination. Note complete demineralization of necrotic area (les). × 8.

FIG. 8. Guinea pig A 1. Note polymorphonuclear leukocytic infiltration of necrotic area. Nissl's stain. × 760.

FIG. 9. Guinea pig A 3. Note ischemic necrosis of ganglion cells and polymorphonuclear infiltration of necrotic area. Hematoxylin-eosin stain. × 760.

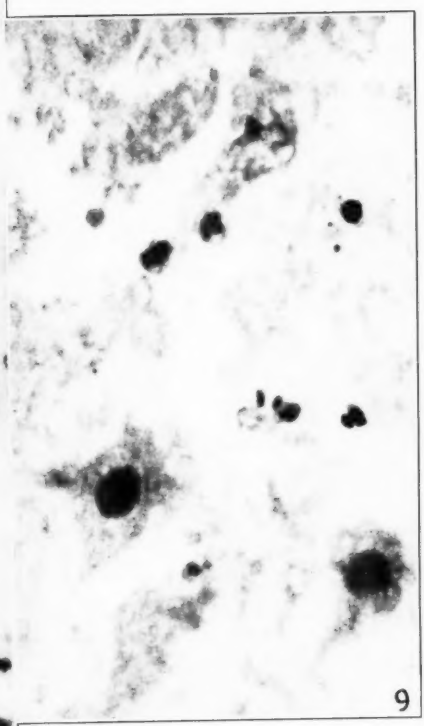
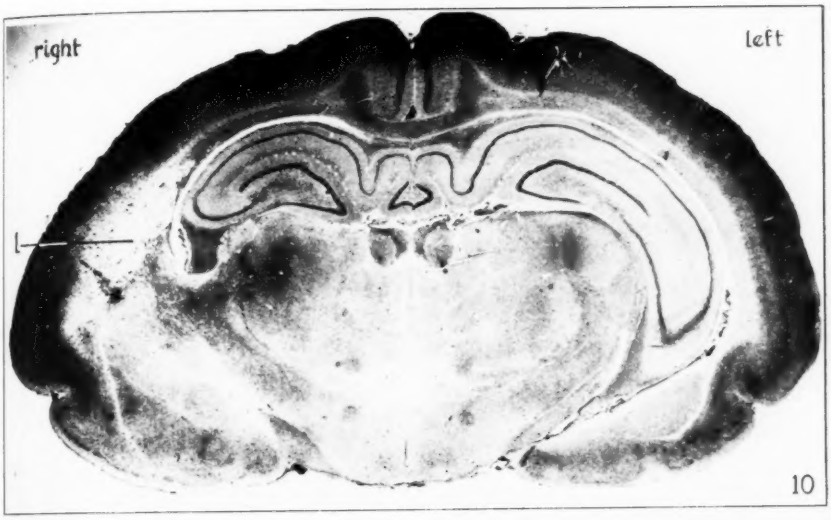



PLATE 37

FIG. 10. Guinea pig A 15. Sensitized, 4 day lesion. Note necrosis (l) in the right parietal lobe. Hematoxylin-eosin stain. × 6.6.

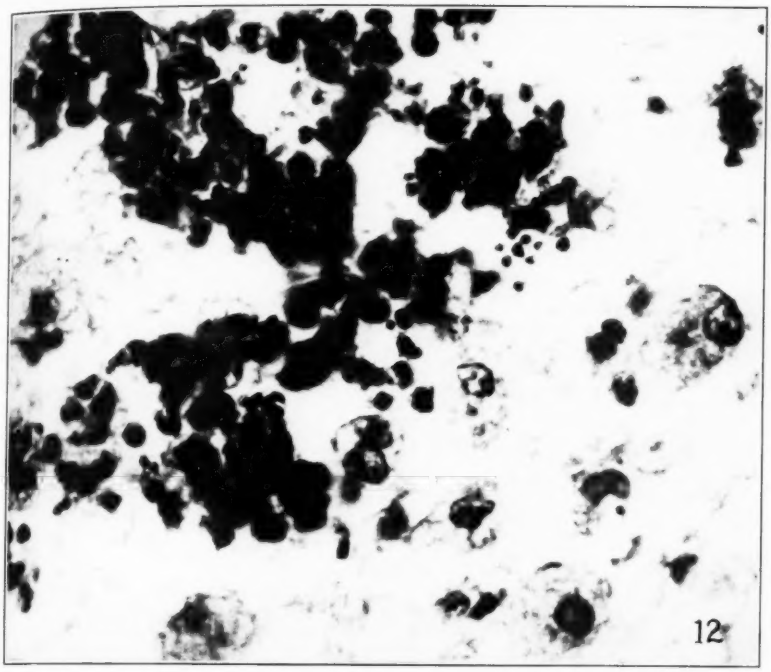
FIG. 11. Guinea pig A 15. Neighboring section to that seen in Fig. 10. Microincineration. Note demineralization of lesion (l). × 6.6.

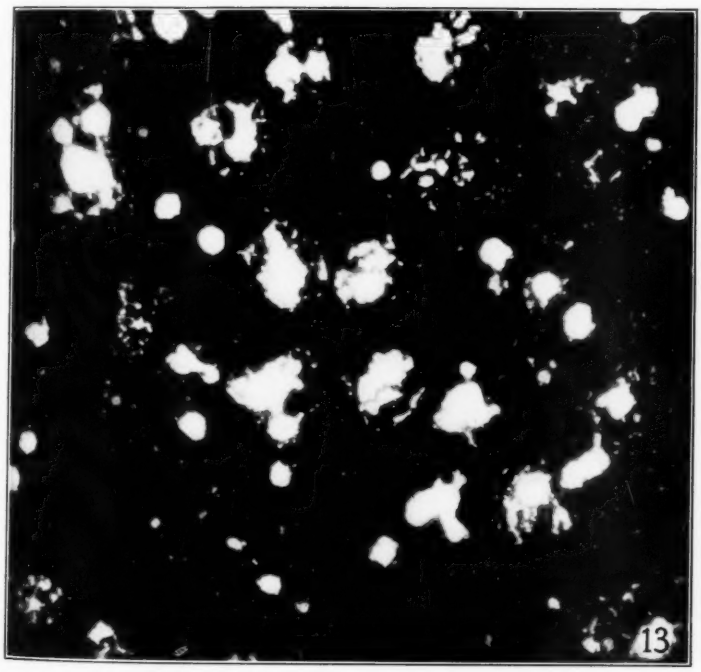



## PLATE 38

FIG. 12. Guinea pig A 15. Note scavenger cells and polymorphonuclears in necrotic area. Masson's trichrome stain. × 760.

FIG. 13. Guinea pig A 15. From incinerated neighboring section to that seen in Fig. 12. Darkfield illumination. Note mineral deposit in scavenger cells, part of which is iron oxide. × 760.



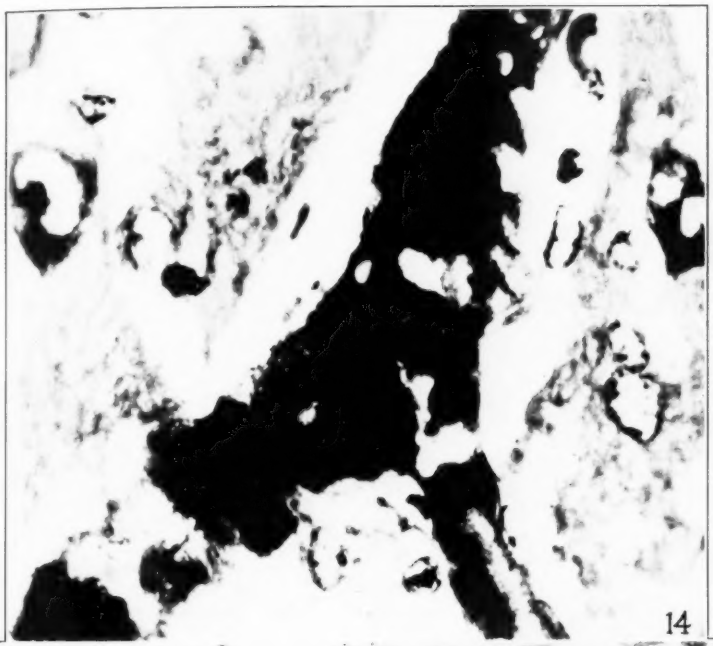




Alexander and Campbell Anaphylactic Lesions in Brain

PLATE 39

FIG. 14. Guinea pig A 21. Sensitized, 7 day lesion. Thrombosed vessel from lesion. Masson's stain. × 1000.

FIG. 15. Guinea pig A 21. Note gliosis: microglial and oligodendroglial cells and astrocytes. Nissl's stain. × 760.



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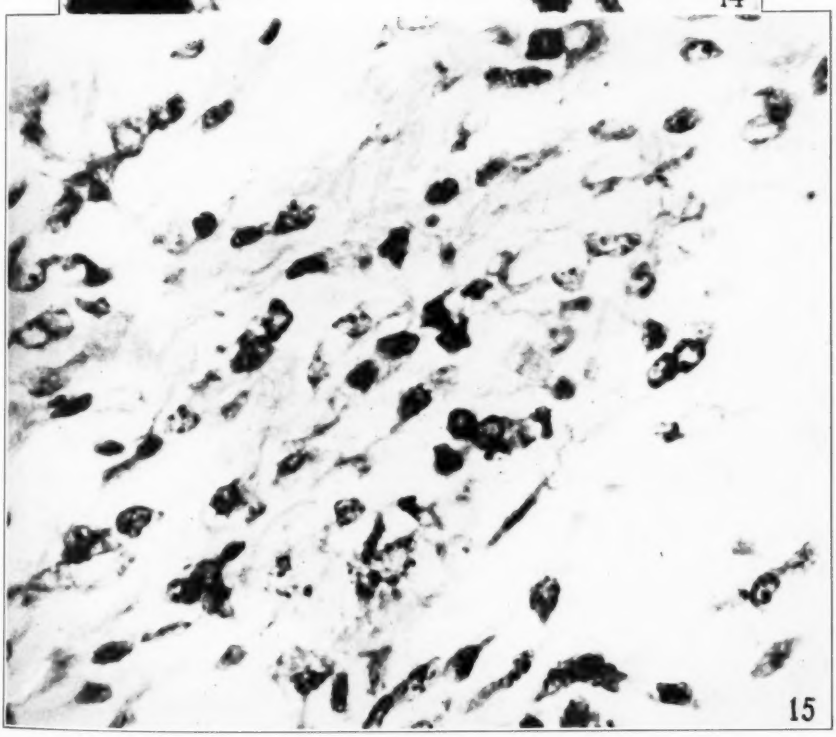


15

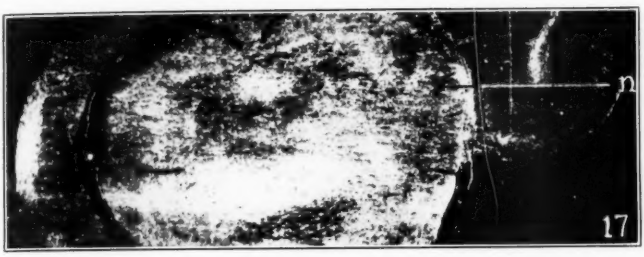
Alexander and Campbell Anaphylactic Lesions in Brain

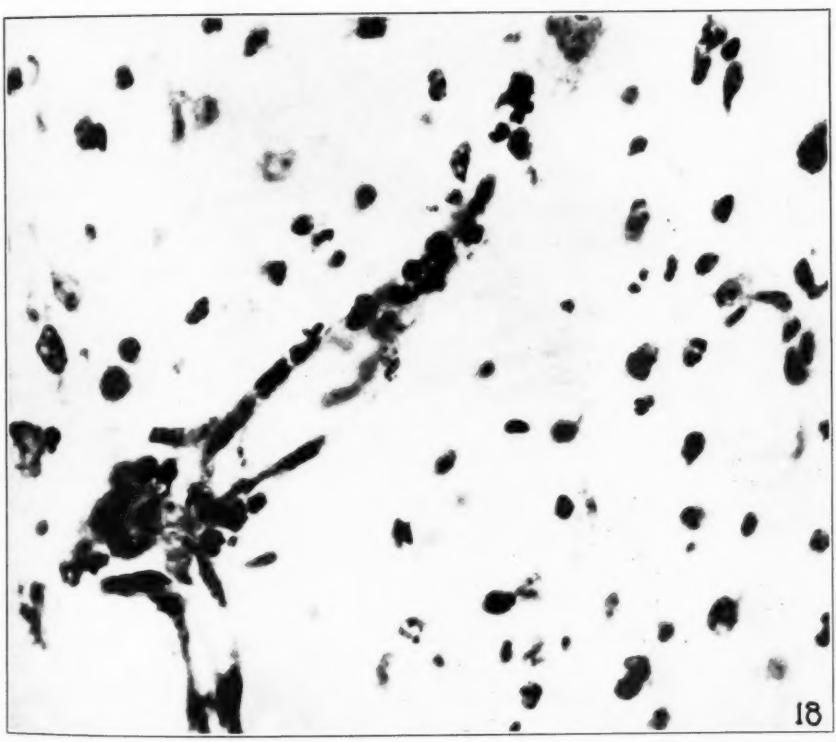
PLATE 40

FIG. 16. Guinea pig A 4. Non-sensitized, 30 hour lesion. Note hemorrhagic streak (h) in right midbrain and small necrotic areas immediately around streak, one of them marked "n". Heidenhain's iron hematoxylin stain. ×8.

FIG. 17. Guinea pig A 4. Neighboring section to that seen in Fig. 16. Micro-incineration, oblique transillumination. Note small areas of demineralization corresponding to the necrosis (n). × 8.

FIG. 18. Guinea pig A 6. Non-sensitized, 30 hour lesion. Polymorphonuclear leukocytic infiltration of lesion. Nissl's stain. × 760.

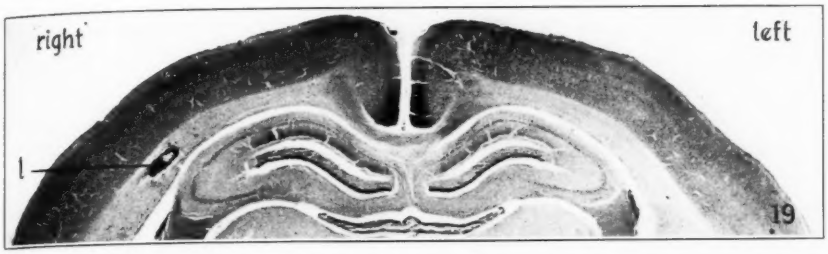

Alexander and Campbell Anaphylactic Lesions in Brain

PLATE 41

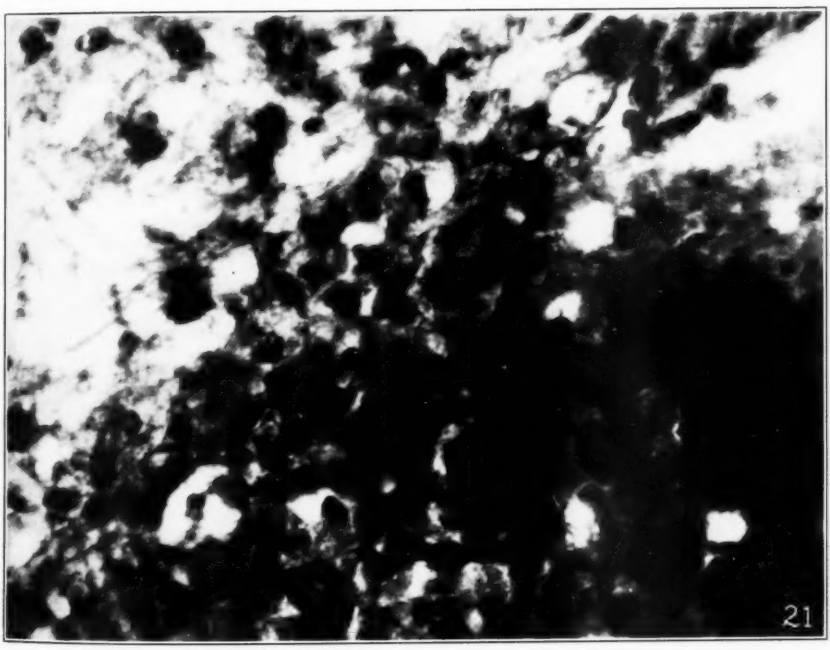
FIG. 19. Guinea pig A 16. Non-sensitized, 4 day lesion. Note hemorrhagic cyst (l) in right parietal lobe, with slight surrounding necrosis. × 6.6.

FIG. 20. Guinea pig A 16. Neighboring section to that seen in Fig. 19. Micro-incinerated, oblique transillumination. Note granular mineral in hemorrhage (l) (part of it iron oxide) and small surrounding zone of demineralization. × 6.6.

FIG. 21. Guinea pig A 16. Non-sensitized, 4 day lesion. Edge of hemorrhagic cyst, showing scavenger cells among erythrocytes in cyst and gliosis (microglia, oligodendroglia cells and astrocytes) around cyst. × 633.








Alexander and Campbell Anaphylactic Lesions in Brain

PLATE 42

FIG. 22. Guinea pig B 3. Sensitized, 24 hour lesion. Section 200 $\mu$ thick, Lepehne-Pickworth stain. Note lesion in central white matter and striatum. It shows central mass of hemorrhage, surrounded by satellite perivascular hemorrhage, outside which is an anemic zone, particularly notable at the upper pole where it extends into the cortex. $\times$ 8.

FIG. 23. Guinea pig B 10. Non-sensitized, 24 hour lesion. Section 200 $\mu$ thick, Lepehne-Pickworth stain. Note lesion in subcortical white matter. It depicts, as in Fig. 22, a central mass of hemorrhage with satellite perivascular hemorrhages, but the zone of anemia around is much less intense. $\times$ 8.

FIG. 24. Guinea pig B 3. Sensitized, 24 hour lesion. Section 200 $\mu$ thick, Lepehne-Pickworth stain. Note lesion (l) surrounded by anemic zone (a), outside of which the normal vascular pattern (n) is seen. $\times$ 180.

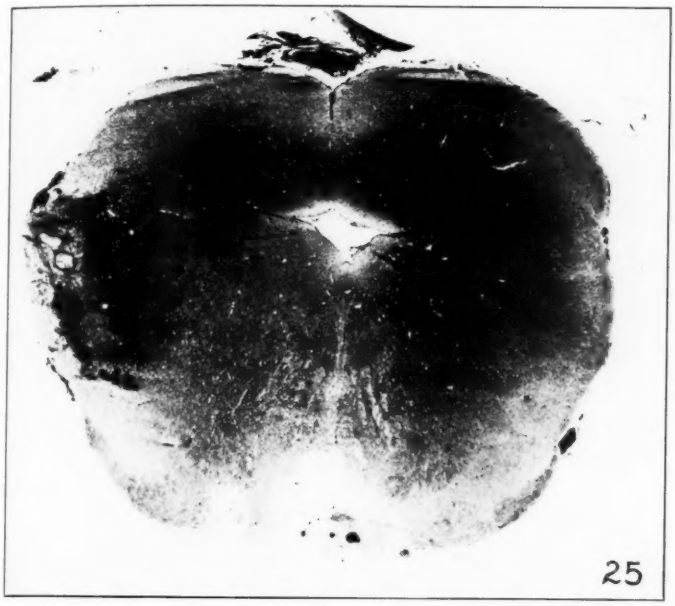



Alexander and Campbell Anaphylactic Lesions in Brain

PLATE 43

FIG. 25. Rabbit 2. Sensitized, 3 day lesion. Note lesion in left midbrain, with hemorrhage and necrosis, part of which shows fibrinous exudate. Masson's trichrome stain. × 8.

FIG. 26. Rabbit 2. Neighboring section to that seen in Fig. 25. Microincineration, oblique transillumination. Note hypermineralization of fibrinous exudate in necrotic area. × 8.



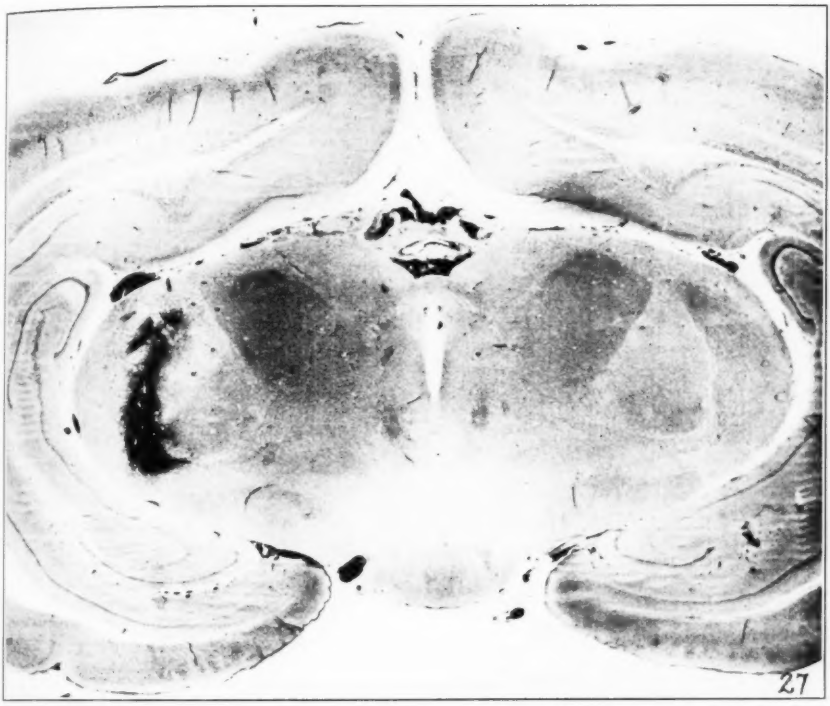


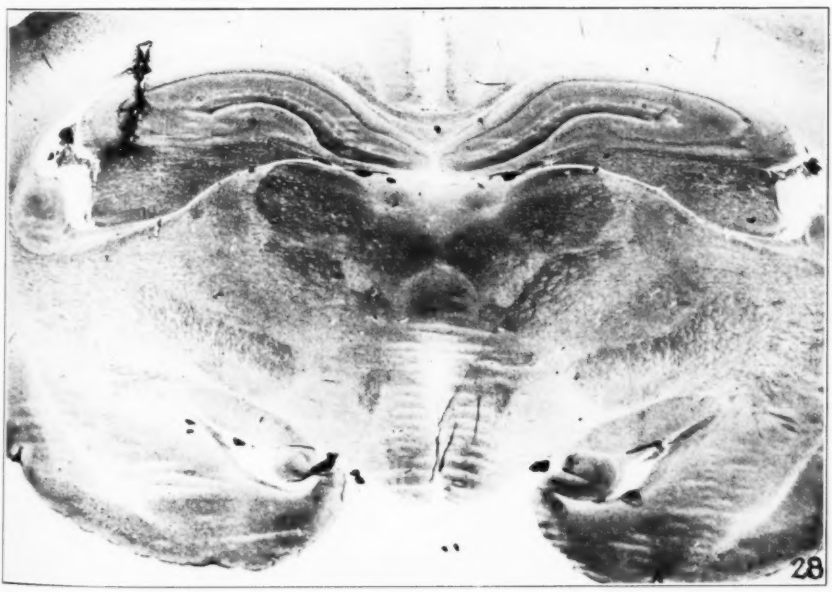
Alexander and Campbell Anaphylactic Lesions in Brain

PLATE 44

FIG. 27. Rabbit 3. Sensitized, 3 day lesion. Note lesion (hemorrhage and necrosis) in left diencephalon (lateral geniculate nucleus, medial geniculate nucleus, and nucleus ventralis B of the optic thalamus). Masson's stain. × 8.

FIG. 28. Rabbit 5. Non-sensitized, 3 day lesion. Note small lesion (hemorrhagic streak) in subcortical white matter and cornu ammonis of left hemisphere. Masson's stain. × 8.



# NUTRITIONAL EDEMA IN THE DOG*

## IV. Peptic Ulcer Produced by the Same Low Protein Diet that Leads to Hypoproteinemia and Edema

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The investigations of Weiss and Aron [1] and the interpretation given by them to the findings of Mann and Williamson [2] by experimental surgery have provoked a new point of view regarding the possible etiology of some cases of peptic ulcer. Briefly it has been shown that dogs develop peptic ulcers with regularity following an operation which involves resection of the duodenum with subsequent occlusion of the upper end and anastomosis of the lower end to the ileum at a point 10–30 cm. above the ileocecal valve, and gastrojejunostomy. By the operation the normal point of entry of bile and pancreatic secretion at the upper end of the small intestine is transferred to the lower ileum. Dogs so treated become rapidly emaciated, develop anemia and bloody diarrhea, and succumb after 4 to 5 weeks. At postmortem examination ulcers are found in the jejunum near the site of anastomosis with the stomach. Weiss and Aron have interpreted their work as indicating that the cause of the ulcers is closely related to defective digestion and absorption of protein. Normally the digestion of protein is not finished in the stomach; complete splitting into amino acids must take place in the small intestine before absorption is possible. Because trypsin from the pancreas is an essential factor in the digestion of protein, the operation described above interferes greatly with the absorption of protein. The strongest argument Weiss and Aron have produced to support this point of view is their finding that the formation of ulcer after the operation on dogs can be prevented by parenteral injections of histidine. Because similar injections of lysine or of tryptophane were without effect, they have come to regard these peptic ulcers as a manifestation of a specific amino acid deficiency.

Following an independent line of investigation Hoelzel and Da

* Received for publication July 28, 1936.

Costa[3] have observed ulcers in the prostomachs of rats maintained on a diet restricted in protein. The finding was interpreted on the basis of the theory that assumes excess acidity to be responsible for the etiology of peptic ulcer. The authors stated that "a protein deficiency or the lack of sufficient acid-binding protein in the diet may be a factor in the etiology." Their work does not touch on the possibility of a specific amino acid deficiency.

For several years the senior author and his associates[4] have been engaged in a study of nutritional edema and hypoproteinemia in the dog. The edema ensues after a period of 1 to 3 months of maintenance on a diet that contains too little protein for nitrogen equilibrium; the hypoproteinemia is progressive from the start of the experiment and affects chiefly the albumin fraction. Since in addition to edema many of the animals have exhibited ulcers of the stomach and duodenum at autopsy and since the relation between protein deficiency and peptic ulcer is attracting general attention, it is desirable to record this feature of the experiments. Of related interest is the fact that Flood and Mullins[5] in producing peptic ulcers in dogs by the operative technic have observed the development of deficits in serum albumin which are similar to those resulting from dietary deprivation of protein.

## Plan of Experiments

*Animals:* The 22 dogs on which this report is based were those that came to autopsy at the end of the experiment. In 12 instances death occurred spontaneously from severe inanition or from terminal infection; in 10 cases the animals were killed by the intracardiac injection of ether after extreme inanition had set in. The average original weight was 17.1 kilos; the average weight at the time of demise was 12.8 kilos.

*Diet:* The method of preparation of the diet and the composition of the diet and salt mixture have been described previously.[4] For convenience the composition of the diet on which most of the dogs were maintained is repeated here.

| | |
|---|---|
| Carrots | 300 gm. |
| Rice | 35 " |
| Lard | 40 " |
| Cod liver oil | 10 " |
| Sugar | 115 " |
| Salt mixture | 5 " |

The amount represented by this mixture was given to each dog daily irrespective of its size. It furnishes 1200 calories and contains 1.23 gm. nitrogen. Five dogs on which metabolism observations were made during subsistence on the diet showed an average daily loss of 1.15 gm. of nitrogen. The low amount of protein is the only important item in which the diet is deficient. The requirement for vitamin A is covered abundantly by the carrots and the cod liver oil; the latter constituent also covers the need for vitamin D. Although carrots furnish some vitamin C this constituent is not necessary for health in dogs. For calculating the vitamin B content of the diet we are indebted to Dr. George Cowgill of New Haven, who found it to be at the threshold of adequacy. To preclude the possibility that an associated B deficiency might be contributing to the results of the experiments the diet of 2 animals (5–69 and 5–70) were supplemented by the daily addition of 0.4 gm. of an extract of rice polishings; in addition as a source of vitamin G 1 of these animals (5–69) received 11 gm. of liver extract daily.* Since both of these animals behaved in all obvious respects like the others and since both exhibited peptic ulcers at autopsy, it is certain that lack of vitamins B or G is not responsible for the postmortem findings. These facts are of some importance since other investigators[6, 7] have reported the presence of ulcers of the stomach and intestine in animals maintained on diets inadequate in vitamin content.

## Pathological Findings

The general behavior of dogs on the low protein diet and the features that characterize the development of hypoproteinemia and edema have been described in another place.[4] Only those findings that bear on a possible relation between peptic ulcer and dietary deficiency of protein will be described here. This purpose in description is best served by a series of brief protocols.

*Dog 2–07* received the diet for 87 days. Death occurred from peritonitis following perforation of duodenal ulcer. There were 2500 cc. of cloudy fluid containing bits of food and fibrin in the peritoneal cavity. In the duodenum be-

* The cod liver oil used in the diet is known commercially as Cod Liver Oil Stearine and was donated by Mr. L. D. Johnson of Mead Johnson and Company. The concentrate of rice polishings is known by the trade name of Ryzamin-B and was furnished by Drs. C. S. Leonard and W. H. Stoner of Burroughs Wellcome and Company. The liver extract was Liver Extract No. 343, furnished through the kindness of Drs. G. H. A. Clowes and G. B. Walden of Eli Lilly and Company.

tween the ampulla and the pylorus were patchy areas where the surface was flattened and deeply discolored. Microscopic examination showed the mucosa in this region to be gangrenous with the process extending into the deeper layers. In the center of one of these areas was a sharply demarcated ulcer, 8 mm. in diameter, extending entirely through the intestinal wall (Fig. 1).

This case was classified as peptic ulcer of the duodenum.

*Dog 5–69* received the diet for 101 days. Death resulted from the intracardiac injection of ether. In the duodenum, 1 cm. below the pylorus, was a well defined, superficial shallow erosion 8 mm. in diameter. The central portion of this erosion, more deeply ulcerated, showed several points of recent hemorrhage. Microscopic section, which unfortunately missed the point of deepest ulceration, showed denudation and necrosis of the mucosa with surrounding areas infiltrated with polymorphonuclear leukocytes.

This case was classified as peptic ulcer of the duodenum.

*Dog 5–70* received the diet for 99 days. Death occurred from inanition. There were two circular, sharply demarcated ulcers, each about 1 cm. in diameter, at the pyloric ring: one extended to the muscle layer; the other was superficial. In addition, three shallow ulcers, 2–3 mm. in diameter, involving the gastric mucosa near the pylorus were present.

This case was classified as peptic ulcer of the stomach.

*Dog 8–40* received the diet for 104 days. Death was caused by hemorrhage from duodenal ulcer. Two sharply defined ulcers were present just below the pyloric ring: one, 8 mm. in diameter, showed evidence of recent bleeding; the other, 1 cm. in diameter, exhibited no hemorrhage. Most of the small intestine was filled with recently clotted blood. The omentum and pancreas were adherent to the duodenum on its external wall. The appearance of a section through the ulcer is shown in Figure 2.

This case was classified as peptic ulcer of the duodenum.

*Dog 4–87* received the diet for 110 days. Death occurred from peritonitis. In the duodenum there was a band of dark discolored mucosa, 2.5 cm. wide, 2 cm. below the pylorus. Two circumscribed ulcers, each 2 by 3 mm., one at the junction of the pylorus and duodenum and the other at the lower edge of the dark band were observed. No site of perforation could be detected.

This case was classified as peptic ulcer of the duodenum.

*Dog 1–91* received the diet for 75 days. Death resulted from the intracardiac injection of ether. There were three sharply defined ulcers present, each about 5 mm. in diameter, one in the fundus of the stomach and two in the duodenum about 2 cm. below the pylorus.

This case was classified as peptic ulcer of the stomach and duodenum.

*Dog 1–92* received the diet for 47 days. Death resulted from the intracardiac injection of ether after the onset of pneumonia. A well demarcated, circular ulcer, about 1 cm. in diameter, involving the pyloric ring, was seen.

This case was classified as peptic ulcer of the stomach.

*Dog 4–51* received the diet for 87 days. Death occurred from inanition. On the lesser curvature of the stomach and near the pylorus there was a deep ulcer, 2 cm. in diameter, with firm cartilage-like edges. Deep congestion of the pyloric

mucosa and a number of small fresh hemorrhages between the pylorus and the ulcer were present. Microscopic examination showed complete destruction of the muscle layer which was replaced by dense scar tissue and scattered areas of cellular infiltration (Fig. 3).

This case was classified as peptic ulcer of the stomach.

*Dog 1–89* received the diet for 123 days. Death occurred from inanition. The mucosa of the upper duodenum was deeply congested but showed no ulceration. A small circular erosion of the gastric mucosa, about 2 mm. in diameter, was present along the greater curvature.

This case was classified as an erosion of the gastric mucosa.

*Dog 2–05* received the diet for 104 days. Death occurred from anemia and inanition. The duodenal mucosa between the pylorus and ampulla was deeply congested. From one spot, 2 mm. in diameter, in this area the mucosa was denuded. Microscopic examination confirmed this finding.

This case was classified as an erosion of the duodenal mucosa.

*Dog 5–65* received the diet for 56 days. Death resulted from the intracardiac injection of ether. The gastric and duodenal mucosa were congested. Along the lesser curvature of the stomach were several pinpoint ulcers and several points of capillary hemorrhage.

This case was classified as an erosion of the gastric mucosa.

*Dog 5–97* received the diet for 85 days. Death resulted from the intracardiac injection of ether. The mucosa of both stomach and small intestine was congested. A number of superficial ulcers of the gastric mucosa, about 2 mm. in diameter, were present near the pylorus. One of these areas, shown in the microscopic section (Fig. 4), extended partially through the mucosa and the surrounding tissues were infiltrated with lymphocytes and plasma cells.

This case was classified as an erosion of the gastric mucosa.

*Dog 1–90* received the diet for 99 days. Death occurred from inanition. The duodenum between the ampulla and pylorus showed deep congestion. There were no gross defects in the mucosa. However, microscopic study revealed a small area associated with hemorrhage from which the mucosa was denuded.

This case was classified as an erosion of the duodenal mucosa.

*Dog 2–3* received the diet for 88 days. Death resulted from the intracardiac injection of ether. There were no lesions of the gastric or duodenal mucosa.

This case was classified as negative for ulcer or erosion.

*Dog 5–89* received the diet for 78 days. Death resulted from the intracardiac injection of ether. The mucosa of the stomach and small intestine was everywhere congested; the congestion was most increased in the regions adjacent to the pylorus. Scattered pinpoint hemorrhages were present in the areas of deeper congestion. Microscopic examination did not reveal defects of the mucosa.

This case was classified as negative for ulcer or erosion.

*Dog 5* received the diet for 59 days. Death resulted from the intracardiac injection of ether. Areas of congestion and edema were present throughout the mucosa of the entire small intestine. There were no points of ulceration or erosion and no hemorrhages.

This case was classified as negative for ulcer or erosion.

*Dog 3–87* received the diet for 79 days. Death occurred from inanition and terminal hypostatic pneumonia. The mucosa in the upper duodenum was deeply congested without erosions or ulcers. Microscopically the capillaries were enormously distended with blood but the surface mucosa was intact.

This case was classified as negative for ulcer or erosion.

*Dog 4–00* received the diet for 86 days. Death occurred from inanition. The mucosa of the duodenum and stomach was normal. The bile duct and pancreatic duct opened separately into the duodenum.

This case was classified as negative for ulcer or erosion.

*Dog 3–67* received the diet for 100 days. Death resulted from the intracardiac injection of ether. Except for intense jaundice there was no pathological change in the stomach or intestine.

This case was classified as negative for ulcer or erosion.

*Dog 2–93* received the diet for 87 days. Death resulted from inanition and pneumonia. The mucosa of the stomach and intestine was normal.

This case was classified as negative for ulcer or erosion.

*Dog 8–38* received the diet for 78 days. Death resulted from inanition. The autopsy notes contain no reference to the stomach or intestine. Presumably these structures were normal.

This case was classified as negative for ulcer or erosion.

*Dog 2–06* received the diet for 135 days. Death resulted from the intracardiac injection of ether after extreme inanition had set in. The stomach contained a ball of matted hair which weighed 64 gm. Although the epithelium of the duodenum and pyloric end of the stomach was moderately edematous there was neither macroscopic nor microscopic evidence of ulceration. This finding has collateral interest since Pappenheimer and Larimore[8] have reported that hair is an inciting cause of ulcers in the esophageal end of the stomach of rats maintained on a deficient diet.

This case was classified as negative for ulcer or erosion.

Tabulation of the foregoing protocols shows that among the 22 dogs there were 8, or 36 per cent, that developed true peptic ulcer on the diet; 5, or 23 per cent, that exhibited superficial erosions of the gastric or duodenal mucosa; and 9, or 41 per cent, in which neither ulcers nor erosions were found.

## Comment

Since this paper has no other purpose than to place on record an incidental finding in the course of another investigation, detailed discussion will be omitted. It is apparent that the findings permit of more than one interpretation. Accessory data concerning gastric acidity, gastric and duodenal enzymes, and gastro-intestinal motility were not obtained. In their absence speculation as to the

nature of the relation between peptic ulcer and a protein-deficient diet must be withheld.

An attempt to correlate the occurrence or non-occurrence of ulcer with other data in our possession has yielded no clear-cut information. The length of time on the diet was apparently not a deciding factor; the 8 dogs that developed ulcers received the diet for an average of 89 days, and the 14 dogs without ulcers were on the diet for an average of 90 days. The degree of emaciation, as gauged by the percentage loss in weight, seemed to be irrelevant; the animals developing ulcers exhibited an average loss in weight of 22 per cent, and the animals without ulcers showed an average weight loss of 27 per cent. The degree of anemia as estimated from hematocrit readings failed to shed additional light on the etiology; in the ulcer group the average relative red cell volume was 31 per cent, and in the group without ulcer it was 34 per cent. The degree of depletion of the serum albumin alone suggested the possibility of a positive correlation: in the ulcer group the average albumin concentration at the time of last analysis was 1.16 gm. per cent; in the group without ulcer it was 1.51 gm. per cent. However, the individual levels varied widely, there was considerably overlapping between the two groups, and the difference is without statistical significance.

## Summary

The development of peptic ulcers of the stomach and duodenum in dogs maintained on a diet deficient in protein is described. Among 22 animals that received the diet for an average period of 90 days there were 8, or 36 per cent, that exhibited true peptic ulcers at autopsy. Of the remaining 14 there were 5, or 23 per cent, that showed erosions of gastric or duodenal epithelium without true ulcer formation.

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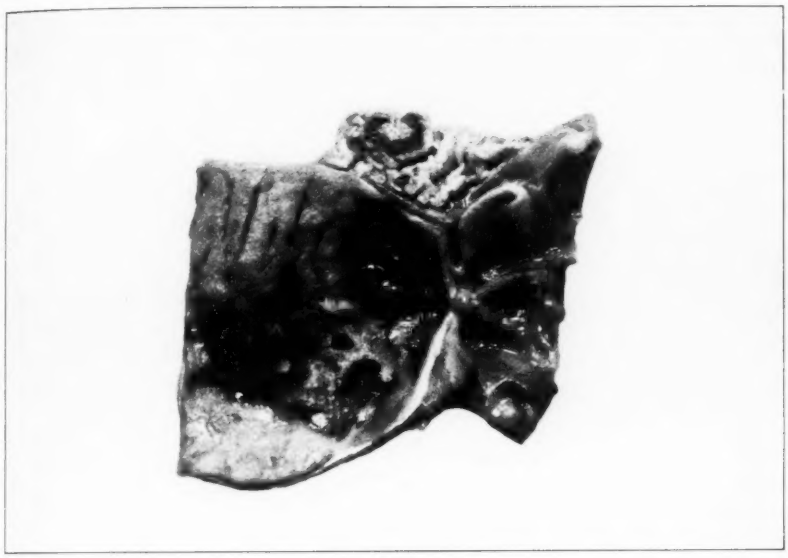
## DESCRIPTION OF PLATES

### PLATE 45

FIG. 1. Dog 2–07. Perforating ulcer of the duodenum with discoloration of the neighboring mucosa.

FIG. 2. Dog 8–40. Ulcer of the duodenum resulting in fatal hemorrhage. × 10.



1

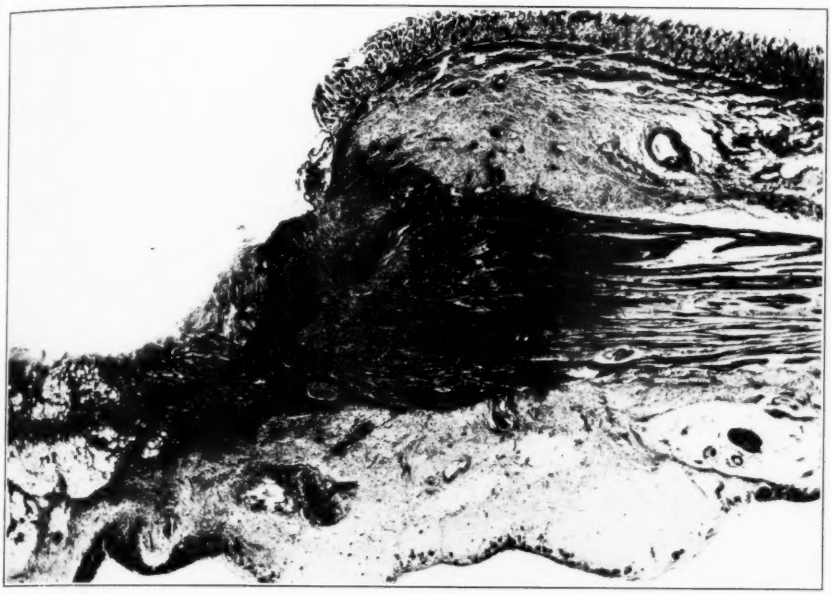
2

Weech and Paige Nutritional Edema in the Dog

PLATE 46

FIG. 3. Dog 4–51. Chronic cicatrizing ulcer of the stomach. × 8.

FIG. 4. Dog 5–07. Superficial erosion of the gastric mucosa. × 8.



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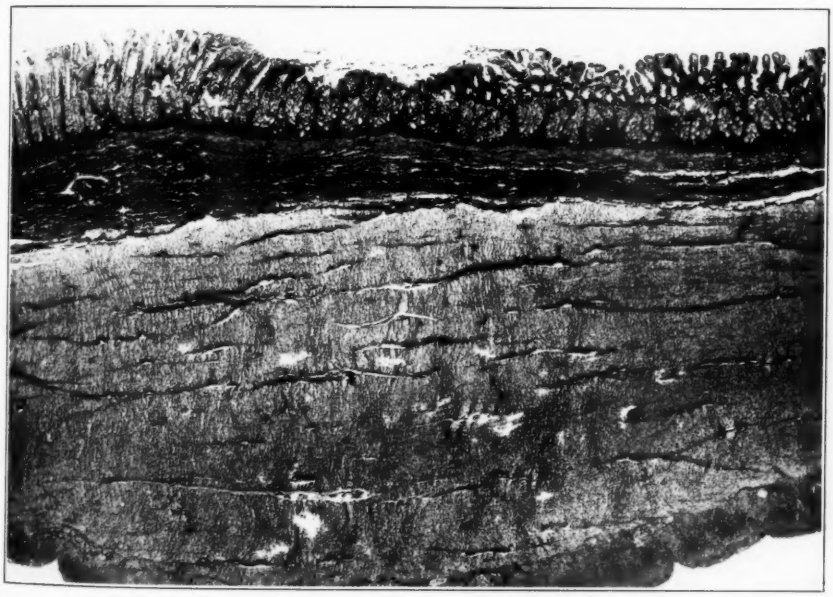

4

Weech and Paige Nutritional Edema in the Dog

# THE SIGNIFICANCE OF MYELIN SHEATH DEGENERATION AND ITS RELATION TO INCOORDINATION *

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A disease occurring in swine designated as posterior paralysis and accompanied by myelin sheath degeneration of the cord and the brachial and sciatic nerves was reported from this Institute by Wehrbein[1] in 1916. The condition is first manifested by a slight posterior weaving gait and incoordination. The incoordination progresses until the affected animals are unable to stand on their hind legs, which are dragged while they pull themselves along with the forelegs. In severe advanced cases the incoordination may become general and involve all four extremities. Bacteriological examinations are negative. Emulsions made from the brain, cord, nerves, heart's blood, bone marrow, spleen and other organs of affected animals will not produce the disease when introduced by various routes into healthy animals. Microscopic preparations of various organs, including the nervous system, stained by hematoxylin and eosin show no specific changes. The only change found consists of degeneration of the myelin sheaths, as demonstrated by the Marchi method for the determination of fat. Later Hughes, Lienhardt and Aubel[2] produced incoordination in swine, with myelin sheath degeneration, by feeding rations designed for the deficiency of vitamin A (white corn, tankage and bone meal).

Biester and Murray[3] found nerve degeneration a constant lesion in field cases and in experimental swine fed rations low in vitamin A. Elder,[4] working with swine, found that rations considered adequate in vitamin A produced myelin degeneration of the nerves. Using rats as experimental animals Zimmerman[5] reported degeneration of the myelin sheaths of the brachial and sciatic nerves but not of the optic nerve.

Zimmerman and Burack[6] demonstrated demyelinization of the nerves of dogs deficient in the vitamin B complex. Further studies by the same authors[7] established that demyelinization of the nerves of dogs could be produced by diets deficient in $B_2$ or G. Prickett[8]

* Received for publication August 12, 1936.

found nerves from rats fed a restricted diet stained with osmic acid, whether receiving vitamins B and G or not. Crane-Lille and Rhoads[9] report myelin degeneration in dogs fed a pellagra-producing diet. Campos, Campos and Maffei[10] report the absence of nerve lesions in experimental vitamin B deficiency in rats.

The similarity of the lesions reported produced by deficiency in vitamin A or the vitamin B complex in different species of animals seemed to indicate that the significance of the pathological lesions of the nerves and cord in these conditions is not clearly understood.

TABLE I

*Data on Field Cases of Swine Showing Posterior Paralysis*

| Pig No. | Farm | Age | Incoordination | Myelin degeneration | | |
|---|---|---|---|---|---|---|
| | | | | Sciatic | Brachial | Cord |
| | | *mos.* | | | | |
| 489 | 1 | 3 | Advanced | + | + | + |
| 490 | 1 | 3 | " | T | | |
| 496 | 2 | 2½ | " | + | + | + |
| 497 | 3 | 4 | " | + | | + |
| 500 | 4 | 4 | Slight | + | T | + |
| 588 | 5 | 6 | Advanced | + | + | + |
| 589 | 5 | 6 | " | + | | + |
| 537 | 6 | 7 | " | + | + | + |
| 551 | 6 | 9 | " | T | T | + |
| 553 | 6 | 9½ | Complete | T | T | + |
| | | *Controls* | | | | |
| 499 | Institute | 4½ | — | — | — | — |
| 498 | " | 4½ | — | — | — | — |
| X | " | Various | — | — | — | — |

T = Trace.

This report will be confined to a discussion of the myelin degeneration of the cord, sciatic and brachial nerves of swine restricted to a certain dietary regimen known to be deficient in vitamin A and $B_1$, as well as one containing A and $B_1$. Details of the vitamin A and $B_1$ experiments will be reported later.

In Table I are shown the results obtained on a few field cases of posterior paralysis in swine. At the time these cases were investigated the presence of myelin degeneration of the cord was considered diagnostic for vitamin A deficiency. Control swine raised

on the Institute farm and fed the stock diet with pasture in season have never shown either incoordination or myelin degeneration.

Table II shows the results of a study of treated pigs from a field outbreak of so-called posterior paralysis. Animals exhibiting the

TABLE II

*Effect of Feeding Vitamin A-Containing Supplements to Field Cases of Swine*

BASAL DIET*

| | | | |
|---|---|---|---|
| Alfalfa meal | 1½ | parts | fed *ad lib.* twice daily |
| Yellow corn (shelled) | 2 | " | |
| Middlings | 2 | " | |
| Chops (½ corn, ½ oats, ground) | 1 | " | |
| Carrots (ground) | 450 | gm. | fed once daily |
| Whole milk | 3 | qts. | |
| Cod liver oil | 200 | cc. | |

*Treated Swine*

| Pig No. | Age | Incoordination | Myelin degeneration | | | |
|---|---|---|---|---|---|---|
| | | | Sciatic | Brachial | Cord | |
| | *mos.* | | | | | |
| 511 | 8 | Recovered | + | + | + | K* |
| 512 | 8 | " | + | + | + | K* |
| 504 | 12½ | " | + | + | + | K* |

*Untreated Controls*

ENTIRE RATION

| | |
|---|---|
| Yellow corn** | 77% |
| Tankage | 20% |
| Bone meal | 3% |

| | | | | | | |
|---|---|---|---|---|---|---|
| 510 | 8 | + | + | + | + | K |
| 492 | 4 | + | + | | + | D |
| 496 | 3½ | + | + | + | | D |
| 487 | 2½ | + | + | + | + | K |

* A-containing ration (7/28 to 11/28); Pig 504 (11/29/32 to 4/19/33).
** Corn from farm where disease occurred.
K = Killed.
D = Died.

typical symptoms, together with some of the corn from the farm involved, were moved to the laboratory for further study.

The yellow corn fed on this farm was of a poor quality. Its nutritional value, especially the vitamin A content, was questioned because of the conditions under which it was grown, due to a severe drought affecting the area.

The untreated group (Table II) was fed this corn with a supplement of tankage and bone meal. Pigs 492 and 496 died and were immediately autopsied. Typical myelin sheath degeneration was observed. Pigs 487 and 510 were killed to obtain fresh tissues. These also presented degenerated myelin sheaths. Pigs 504, 511 and 512 were placed on a ration that was high in vitamin A and its precursors. In these pigs the symptoms of incoordination disappeared at the end of about 3 months. They were again able to stand and walk (Figs. 1 and 2). The symptoms could be brought back by severe exertion and fatigue but would disappear after rest. The recovered animals were kept on the ration high in vitamin A for an additional month, after which time Pigs 511 and 512 were killed. Severe myelin degeneration was found in these two animals although the clinical symptoms had disappeared (Fig. 3). The factor that corrected the incoordination apparently had no appreciable effect on the myelin degeneration. At this time Pig 504 was placed on a vitamin A-deficient ration for nearly 5 months for the purpose of bringing back the symptoms of incoordination, but without success. On postmortem examination the myelin degeneration was not quite as severe as in Pigs 511 and 512.

The authors are indebted to Prof. V. E. Nelson, of the Department of Chemistry, for assay of the corn from the field cases cited in Table II. The results were not available until the close of the experiment. It showed that the vitamin A content did not differ from that of the corn used on the Institute farm where incoordination appeared only in experimentally produced cases. A consideration of the evidence in this and subsequent experiments in which we were unable to cure the condition by cod liver oil alone suggests that some factor other than vitamin A corrected the incoordination.

A ration known to be deficient in vitamin A, as ascertained by assay on chicks, was fed to a group of pigs from farrowing time until the animals were killed for pathological material. In all cases myelin degeneration was present (Table III). Pig 569 with complete inability to stand or walk on its hind legs was brought indoors and given massive doses of cod liver oil and a vitamin B concentrate. No improvement was noted in 2 months so it was killed. Controls 522, 531 and 532 were fed the same ration as the others but as soon as they developed a stilted gait at 4 months of age yellow corn was substituted for white corn and cod liver oil added to

the diet. The objective was to check and cure the condition in its early stage.

The severity of the incoordination was not correlated with that of

TABLE III

*Data on Swine Fed Diet Deficient in Vitamin A*

BASAL DIET

| | |
|---|---|
| White corn | 78% |
| Tankage | 20% |
| Bone meal | 2% |

| Pig No. | Time on diet | Incoordination | Myelin degeneration | | |
|---|---|---|---|---|---|
| | | | Sciatic | Brachial | Cord |
| | *mos.* | | | | |
| 523 | 9 | Slight | + | | + |
| 524 | 10 | " | ++ | + | + |
| 527 | 12 | " | + | | + |
| 528 | 12 | " | + | + | + |
| 529 | 12 | " | + | + | + |
| 530 | 12 | " | + | + | + |
| 534 | 14 | " | T | T | + |
| 535 | 14 | " | T | T | + |
| 555 | 7½ | " | T | T | + |
| 552 | 8 | Complete | T | T | + |
| *569 | 9½ | " | + | + | + |
| 574 | 9 | None | T | T | + |

*Controls*

BASAL DIET

| | |
|---|---|
| Yellow corn | 78% |
| Tankage | 20% |
| Bone meal | 2% |
| Cod liver oil | 15 cc. per pig daily |

| | | | | | |
|---|---|---|---|---|---|
| 522 | 9 | Stilted gait | + | | + |
| 532 | 12 | " " | + | + | + |
| 531 | 12 | " " | + | + | + |

* Cod liver oil and Harris B concentrate — no effect.
T = Trace.

the myelin degeneration. It is also pointed out that these rations contained adequate vitamin B complex. Tests on day old chicks showed that corn supplies sufficient vitamin B complex when fed at a level of 75 per cent of the ration.

The storage of vitamin A was next considered in a group of swine. Sows were placed on white corn, skim milk powder and bone meal from 1 to 3 months before breeding. Both white corn and the skim milk powder had been assayed on chicks and found to be very low in vitamin A but adequate in vitamin $B_1$. The pigs of this group (Table IV) developed symptoms of incoordination very early in life,

TABLE IV

*Data on Pigs from Sows Fed 1–3 Months Prior to Breeding on 79% White Corn, 20% Skim Milk and 1% Bone Meal*

| Pig No. | Age | Incoordination | Myelin degeneration | | |
|---|---|---|---|---|---|
| | | | Sciatic | Brachial | Cord |
| | mos. | | | | |
| 655 | 3 | Advanced | T | T | + D |
| 654 | 3 | " | T | T | + D |
| 656 | 3 | " | T | T | D |
| 646 | 4 | " | T | T | D |
| 647 | 4 | " | T | T | + K |
| 657 | 7 | " | T | T | + K |
| *Skim Milk Powder ad lib.* | | | | | |
| 671 | 8 | Recovered | T | T | T K |
| 665 | 10 | " | T | T | + K |
| 743 | 12 | " | ? | ? | ? K |
| 744 | 12 | " | ? | ? | ? K |
| *Controls Received Yellow Corn + Cod Liver Oil After Early Symptoms* | | | | | |
| 748 | 13 | None | T | T | + K |
| 749 | 13 | " | T | T | + K |

T = Trace.
D = Died.
K = Killed.

before weaning in many instances. The severity of the incoordination increased rapidly in this group, some individuals succumbing as early as 3 months of age. These pigs, unlike those shown in Table III, showed severe incoordination but only mild nerve degeneration and less severe changes in the cord. Pigs 671, 665, 743 and 744 were given skim milk powder *ad lib.* at the age of 4 months. After 3 months the ration was changed to include equal amounts of ground

white corn and skim milk powder. Pigs 748 and 749 were fed yellow corn and cod liver oil at 2½ months of age.

Pigs 743 and 744 (Table IV) fed large amounts of skim milk powder until 12 months of age revealed no changes in the myelin sheaths by means of the Marchi method. The fat adjacent to the nerves also failed to react. A failure in technic was considered. The blocks were blackened but when microtome sections were made these appeared yellowish gray. The remaining portion of the Müller's fluid-osmic acid mixture was used on control material which reacted in the conventional manner by blackening fat and degenerated myelin. Investigations are underway to determine the presence of some factor in skim milk powder that may be responsible for this behavior. It should also be noted that only traces of degeneration could be demonstrated in Pigs 671 and 665 fed large quantities of skim milk powder for 8 and 10 months respectively.

In Figures 4, 5 and 6 are shown cords of pigs showing degeneration on low vitamin A rations, adequate vitamin A rations and a normal control.

Grains are known to contain vitamin B but since many of them are stored long periods and often harvested in an immature state the possibility of a vitamin B deficiency as the cause of demyelinization of the nerves of swine was considered.

The presence of myelin degeneration after recovery from incoordination (Table II) and the findings in rats by Zimmerman suggested the need of some data for comparative purposes on the effect of vitamin $B_1$-free rations on swine. In attempting to evaluate a change such as myelin degeneration it should be realized that basal rations used in nutritional studies are extremely limited and artificial. In an effort to control a given vitamin we believe unknown factors are introduced.

Normal swine of various sizes were fed diets previously assayed on chicks for vitamin $B_1$. Any rations fed chicks, 24 hours after hatching, which died of polyneuritis within 7 to 12 days, were considered deficient in $B_1$. In Table V are shown the results of the effect of the presence or absence of $B_1$ on myelin degeneration of the nerves of swine on restricted diets. The type of degeneration is identical with that shown in Figures 1 and 2. These results show definitely that vitamin $B_1$ does not play a part in the production of these lesions since both the controls and the B deficient animals show degenera-

tion. Since cod liver oil was fed to all pigs on the $B_1$ experiment, vitamin A was eliminated as a causal factor.

Evidence obtained from other species has indicated that diets lacking in some unknown factor, exclusive of vitamins A and $B_1$, will produce myelin degeneration. Biester, Greenwood and Nelson,[11]

TABLE V

*Vitamin $B_1$-Free Rations Fed Swine**

Polished rice + washed casein 10%, tankage 10%
Polished rice + tankage 20%
Polished rice + washed casein 10–20%**

| TISSUES | INCOORDINATION | MYELIN DEGENERATION |
|---|---|---|
| No. pigs 7 | None | Spinal cord 100% |

*Vitamin $B_1$-Containing Rations**

Polished rice + untreated skim milk powder 10%, tankage 10%
Polished rice + tankage 20% + yeast 2.1%
Polished rice + washed casein + yeast 2.1%

| TISSUES | INCOORDINATION | MYELIN DEGENERATION |
|---|---|---|
| No. pigs 5 | None | Spinal cord 100% |

* Each basal ration included bone meal 1%, NaCl 0.3%, and 15 cc. cod liver oil per pig. $FeCl_3$ was fed to groups not receiving tankage.
** 10% fed during first 104 days.

using dogs during the course of studies on fluorine toxicology, found severe myelin degeneration in the spinal cords of both fluorine-fed and control animals (Fig. 7).

During the first 4 months the dogs received 180 cc. of whole milk and a commercial dog preparation containing 19 per cent digestible protein. Vitamins A, B, D and G were amply supplied. The vitamin C content of the ration was low. From 4 months of age until destruction the dogs received a daily ration patterned after that of Mellanby, which consisted of the following:

| | |
|---|---|
| Yellow corn | 30 parts |
| Hulled oats | 30 " |
| Ideal dog food (commercial) | 23 " |
| Wheat germ meal | 5 " |
| Skim milk powder | 10 " |
| Cod liver oil (Squibb) | 1 " |
| Sodium chloride (0.01 gm. NaI per 100 gm.) and | 1 " |
| Whole milk | 180 cc. |

The yellow corn and hulled oats were moistened, autoclaved 1½ hours at 15 pounds pressure and then dried. This was done to render them more palatable before incorporation in the mixture. The commercial dog food contained approximately 12 per cent protein. This ration included sufficient quantities of vitamins A, B, D and G.

The dogs fed the above ration showed, in addition to the spinal cord lesions, fatty degeneration of the renal epithelium in the medullary rays confined chiefly to the spiral convoluted tubules. This characteristic distribution could be seen plainly without magnification in the slides prepared by the Marchi method. These dogs manifested no incoordination or other clinical symptoms. Blood and urine examinations by standard chemical methods were negative for nephritis.

Day old chicks were kept on the same rations as the swine. Although the chicks succumbed or were moribund as a result of A or $B_1$ deficiencies respectively, myelin degeneration in the sciatic nerves was absent in most cases while in a few animals a few fibers were affected. The absence of or relatively slight nerve lesions found in these chicks was ascribed to the short duration of the experiments. This may also account for the results of Campos, Campos and Maffei,[10] who fed rats vitamin-deficient rations for relatively short periods of time. The high protein rations (42 per cent casein) used by these authors may be a factor, since unconfirmed results obtained in one of our experiments indicate that *ad lib.* feeding of skim milk powder will cure incoordination and produce a regeneration of the myelin sheaths (Pigs 671, 665, 743, 744 (Table IV)). Further experimentation on this point is underway. Field cases of incoordination were cured by *ad lib.* feeding of skim milk powder. Unfortunately tissues from these cases were not available.

## Summary and Conclusions

These experiments suggest that the incoordination and myelin degeneration in the nervous systems of swine are caused by different etiological agents.

Severe myelin degeneration has been produced in the absence of incoordination.

Experiments conducted over a period of 4 years have shown that

neither vitamins A nor B complex are responsible for the myelin degeneration of the spinal cords and peripheral nerves in swine.

Severe myelin degeneration without incoordination was found in dogs fed a ration that included vitamins A, B complex, D and E.

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## DESCRIPTION OF PLATE

### PLATE 47

FIG. 1. Showing affected pigs (Nos. 511, 512 and 504).

FIG. 2. Same pigs 3 months after change of ration.

FIG. 3. Spinal cord from recovered case, Pig. 511. Marchi method. × 400.

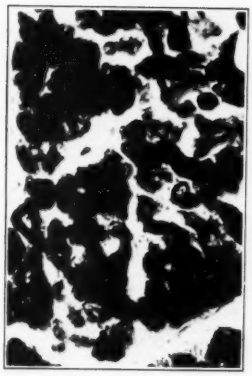
FIG. 4. Spinal cord from pig receiving ration containing B complex but deficient in A. Marchi method. × 400.

FIG. 5. Spinal cord from pig receiving adequate A and B complex. Marchi method. × 400.

FIG. 6. Spinal cord from control pig kept on pasture. Marchi method. × 400.

FIG. 7. Spinal cord from dog receiving vitamins A, B complex, D and E. Marchi method. × 400.

1



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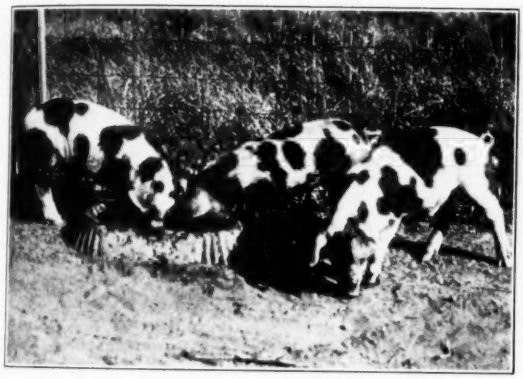

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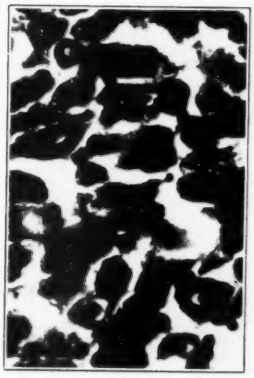

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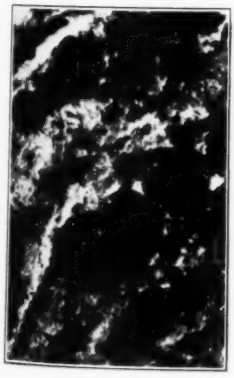

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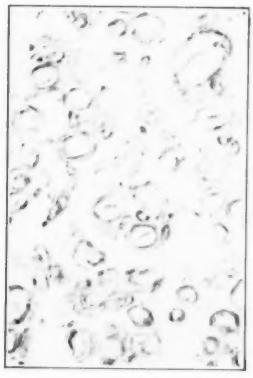
Eveleth and Biester



6

7

Myelin Sheath Degeneration

# MESENTERIC CHYLADENECTASIS*

## Report of a Case

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Although moderate dilatation of lymph node sinuses is commonly seen, extensive dilatation with the formation of multiloculated or single cysts is exceedingly rare. Ectasia of the mesenteric lymph nodes with inspissation of chyle is apparently even more unusual. Von Rokitansky[1] first discussed this condition in 1855. He reported a case in which multiloculated dilatation of the mesenteric lymph nodes was found. Fatty material filled these cystic spaces.

Gjorgjević[2] in 1871 first proposed the term lymphadenectasis to describe dilatation of lymph nodes. Odenius[3] classified cystic lymphangioma separately from simple dilatation of lymph node sinuses. This latter condition was termed lymphadenocele, while development of a central cyst by gradual distention was called simple cystic metamorphosis.

Many theories to explain the dilatation of lymph nodes and lymphatics have been proposed. The mechanical theory suggested by von Rokitansky[1] and Killian[4] emphasized passive formation of dilatation due to obstruction of lymph vessels. Klebs[5] mentioned increased lymph production peripherally. Obstruction of the thoracic duct in the etiology has been advocated by Enzmann,[6] Killian,[4] and Virchow.[7] So-called infarction of efferent vessels caused by inspissated chyle has been suggested by von Rokitansky[1] and Spaeth.[8] Gross[9] produced congestion of chyle by tying the thoracic duct and the iliac vein. He believed that a congestion of circulation was also necessary. Naumann[10] spoke of a chronic desquamative lymphangitis as a possible cause of mesenteric cysts. Orth[11] attributed local disturbances of lymph circulation to inflammatory changes.

The dilatation of lymph channels and chylous retention cysts has even been linked with neoplasm. In the cases of lymphangioma and chylangioma, Wegner[12] and Sudhoff[13] have assumed that first re-

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tention congestion may occur, followed by irritation or by trauma, and the result is the formation of a neoplasm, chylangioma or lymphangioma. Sudhoff[13] went even farther and assumed that both retention cysts and true chylangioma might occur in the same patient simultaneously. However, Swartley[14] believed that cystic malignant disease of the mesentery, as suggested in the classification of Carter,[15] was not tenable. He assumed malignant changes occurred in already existing cysts.

The case herein reported does not fall in the group of true neoplasms or the classification of mesenteric cysts arising from embryonic rests emphasized by Dowd,[16] Ney and Wilkinson,[17] and Swartley.[14] The cystic changes of the lymph nodes more closely simulated the lesions originally reported by von Rokitansky.[1]

Clinically this case is of interest because of the difficulty of diagnosis. The outstanding symptoms of ravenous appetite, somnolence and emaciation were also noted by Wilson[18] in his report of a large chylous cyst.

## Report of Case

*Clinical History:* The patient, a white Polish farmer, aged 60 years, was admitted to the hospital with the complaint of vomiting, diarrhea, and pain in the epigastrium for 2 months. No history of any relative past illnesses could be obtained with the exception of a similar attack 1 year previous. The latter assertion was made on only one occasion and could not be confirmed.

Physical examination revealed an extremely emaciated white male, mentally confused. The only positive findings consisted of oral sepsis, a few râles heard over the base of the lungs and poor heart tones. Palpation of the abdomen and rectal examination revealed nothing. The blood pressure was 110/70. The hemoglobin was 60 per cent by Sahli, the red cell count 3,250,000, the white cell count 6800, of which 73 per cent were polymorphonuclears and 27 per cent lymphocytes. The blood chemistry showed a blood sugar of 35 mg. after the specimen had been in the icebox overnight. The chemistry was otherwise negative. X-ray study of chest and gastro-intestinal tract was not diagnostic.

The patient's condition became progressively worse, although he made no definite complaints. He became extremely weak and hypotension developed. His appetite was good but he was somnolent.

The temperature never exceeded normal, except during the period just before death, and was for the most part subnormal, around 97° F. The pulse ranged from 70 to 80.

Mental confusion developed, then coma, and the patient died 7 weeks after entering the hospital.

## Postmortem Examination

The body was that of an extremely emaciated white male, 60 years of age, 165 cm. in length, weighing 90 pounds. The skin was pale.

*Thorax:* There were about 200 cc. of straw colored fluid containing small flakes and strings of fibrin in the left pleural space. The lungs were intensely edematous with passive hyperemia posteriorly. There were also poorly outlined, irregular areas of slightly increased density in these congested portions of the lungs. The heart showed adherence of the two layers of the pericardium. The myocardium was a dark brown color, extremely flaccid with a peculiar gelatinous appearance and almost diffluent in consistence.

*Abdomen:* The liver and spleen showed moderate chronic passive congestion. The gastro-intestinal tract was edematous and congested throughout. The pancreas exhibited an increase in density, and a moderate nephrosclerosis of both kidneys was found.

*Mesentery:* All the mesenteric lymph nodes were enlarged, and ranged in size from 0.8 to 3 cm. in diameter. They were firm, smooth, discreet, and on section all exhibited a cystic spongy appearance. The same characteristics were apparent in even the smallest mesenteric lymph nodes. The cut surface was of a cream white color with a fatty material exuding from the small cystic spaces. This same type of lymph node enlargement was likewise found retroperitoneally around the pancreas.

The head and neck were not dissected.

*Anatomical Diagnoses:* Mesenteric chyladenectasis, chronic and acute mesenteric and retroperitoneal lymphadenitis and lymphangitis, chronic pancreatitis with islet hyperplasia, moderate generalized arteriosclerosis, nephrosclerosis, brown atrophy of heart, chronic passive hyperemia of liver, spleen, and gastro-intestinal tract, chronic fibrous adhesive pericarditis, hypostatic bronchopneumonia, and acute serofibrinous pleuritis.

## Histological Findings

On microscopic examination the spongy cystic structure of the lymph node resolved itself into dilated, tortuous communicating spaces, entirely filled with homogeneous lipoid material (Fig. 1). The dilated spaces had no consistent cell lining, but instead were limited by the reticulum fibers and cells of the lymph node stroma. However, there were fairly frequent giant cells which were occasionally sufficiently numerous to give the semblance of a complete lining. These cells appeared actively phagocytic with numerous fine particles of fat in their cytoplasm, and not infrequently engulfed

entire leukocytes. In structure the giant cells were of the usual foreign body type with somewhat oval shaped nuclei having no characteristic arrangement.

The lymph node stroma consisted of the usual recticulum showing numerous bands of thickening and areas of fibrosis. Thickening of the capsule was likewise found in all the involved lymph nodes. Not infrequently areas of hyalinization of the reticulum were encountered. This process was most common in the group of lymph nodes adjacent to the pancreas. In these, too, the lymph nodules often exhibited central hyalinization, and in some instances complete obliteration of the nodules resulted.

The cellular elements of the lymph nodes were interesting because of their variety. In addition to the giant cells already noted there was an increase in the number of the reticulum cells, likewise most notable in the areas close to the dilated sinuses (Fig. 2). Various stages of differentiation from the small simple type with oval, chromatin-poor nuclei and scanty, somewhat elongated, poorly defined cytoplasm, all the way up to the giant cell were found. The intermediate forms exhibited widening of the cytoplasm with definite cytoplasmic boundaries and a slightly larger round nucleus with occasional prominent nucleoli. These more differentiated forms of reticulum cells contained many fine fat droplets in their cytoplasm. In addition fairly numerous plasma cells and tissue mast cells were demonstrated. The lymphocytes for the most part were arranged diffusely and in small, poorly outlined collections. This breaking up of the lymph nodule architecture was, of course, more pronounced in the nodes showing the greatest degree of ectasia.

Scattered polymorphonuclear neutrophiles were found in all lymph nodes but were relatively scarce in those of the lower part of the mesentery, and much more numerous in the lymph nodes in the pancreatic group. In the latter instance actual plugging of the lymphatic vessels and parts of the cortical sinuses with leukocytes could be seen.

A search for microorganisms demonstrated the presence of Gram-positive cocci in chains in the lymphatic vessels and lymph nodes. Their number ran parallel with the degree of polymorphonuclear leukocytic infiltration. In the lower mesenteric nodes small numbers of these organisms were found, most frequently located in the perivascular lymph spaces.

Examination of the sinus contents showed a homogeneous material which took a uniform red stain with scharlach R. Polariscopic study did not reveal anisotropic characteristics.

No increase of smooth muscle fibers was found in these lymph nodes. The dilated sinuses particularly failed to exhibit an indication of smooth muscle as found in lymph vessel walls.

The loose fibrous tissue of the mesentery adjacent to lymph nodes showed diffusely scattered plasma cells and large numbers of dilated lymph capillaries. The capillaries were made up of flattened endothelial cells only. No mitoses or atypical cytological features were seen. These minute vessels were completely filled with lipoid material identical with that described in the lymph nodes.

*Pancreas:* There was definite increase of the interstitial fibrous tissue, particularly of that around the duct system, with the frequent formation of a partially hyalinized fibrous tissue collar. Polymorphonuclear neutrophiles could be demonstrated in the periductal lymphatics and were found entirely occluding the large lymphatics in the peripheral portions of the pancreas. An unusual feature was the proliferation of the intercalary ducts and the centroacinal cells and an increase in number of large islands. In a few areas where exocrine glandular epithelium was almost entirely replaced by fibrous tissue, a proliferation of islet cells assumed the proportion of an adenomatous type of hyperplasia. Scharlach R preparations showed increased lipoid content in the islet cells, sharply differentiating them from the surrounding parenchyma.

*Intestine:* A definite filling with fat and some distention of the lacteals and associated lymphatics was demonstrated in scharlach R preparations.

Microscopic examination of other organs showed hypostatic bronchopneumonia, moderate nephrosclerosis, and brown atrophy of the heart.

## Discussion

The lymph node lesions in this case appeared to be due to dilatation by chyle, with subsequent breakdown of the emulsion and inspissation. The causative obstruction must have been of a widespread and diffuse character, in view of the rich collaterals in the lymphatic drainage of the mesentery. Such a requirement was provided by the unusual chronic inflammation and fibrosis in the

lymph nodes of the entire mesenteric, peripancreatic and upper retroperitoneal regions.

The attack of abdominal pain 1 year previous to hospitalization may have had a casual relation to the old hyalinized fibrosis of pancreas and lymph nodes. Obliteration of some lymphatic channels probably occurred. However, the more recent active chronic inflammation most likely accounted for the obstruction of the major portion of the lymphatic routes of drainage with consequent sinus dilatation. The possibility that this lymphadenitis in itself permitted the lymph nodes to dilate more readily should also be considered. The acute exudative inflammation of the lymphatics was apparently a terminal process with chylous stasis favoring the rapid spread of the infection.

As to the location and nature of the initial infectious process, one can only speculate. Both the pancreas and the intestine must be considered. Inflammatory changes were more prominent in the proximal group of lymph nodes in the pancreatic region while ectasia was the most evident feature of the mesenteric group. Nevertheless, some attempt at collateral compensation was seen in the presence of small amounts of fat in the peripancreatic lymph node sinuses and lymph vessels.

While somewhat similar gross and histological pictures are found in undoubted true neoplasms, chylangiomas or lymphangiomas, as described by Sudhoff[13] and von Haberer,[19] the case herein reported differed in several important features. The cystic spaces had all the characteristics of lymph node medullary sinuses, were definitely located in lymph nodes and had no true endothelial lining. Furthermore, smooth muscle could not be demonstrated around these cystic channels, as reported by Schmidt,[20] and von Haberer.[19] In addition the age of 60 years is against congenital factors, as in chylangiomas, which are reported most frequently in children and occasionally in young adults. Such cases have been reported by Royster,[21] Sudhoff,[13] Flynn,[22] Lauterburg,[23] Collins and Berdez[24] (Case 1), Harbitz,[25] and Ebhardt.[26] Finally, although there was some dilatation of intestinal lacteals and lymphatics, the typical cystic masses did not involve the intestine at any point.

The addition of chyladenectasis as a separate group to the classification of mesenteric cysts seems justified. Cases of lymph node dilatation resulting from inflammatory and mechanical factors are

probably not of the extreme degree of rarity indicated by a survey of the literature. Some have undoubtedly been reported as chylous mesenteric cysts and some probably as chylangioma or cystic lymphangioma. The second case of Collins and Berdez[24] seems to be one of dilated lymph nodes.

The diagnosis of chyladenectasis must be considered in cases of multiloculated multiple cysts located in the mesentery and not involving the intestine wall, particularly in older adults. The finding of chylous or fatty contents in the dilated lymph node sinuses without a complete endothelial lining, the absence of smooth muscle fibers in the walls of the cystic spaces, and a suitable obstruction to chylous drainage are the main points in diagnosis.

## Summary and Conclusion

A case of mesenteric chyladenectasis is reported.

Microscopic study demonstrated lymph node sinuses dilated by inspissated chyle.

All the unusual cellular elements of lymph nodes were either inflammatory infiltrates, or reactive proliferations, attempting to remove the lipoid material in the sinuses.

The case is reported because it seems to explain the genesis of one type of mesenteric cysts.

Note: The author is indebted to Dr. W. F. Jacobs, Buffalo City Hospital, for permission to report this case.

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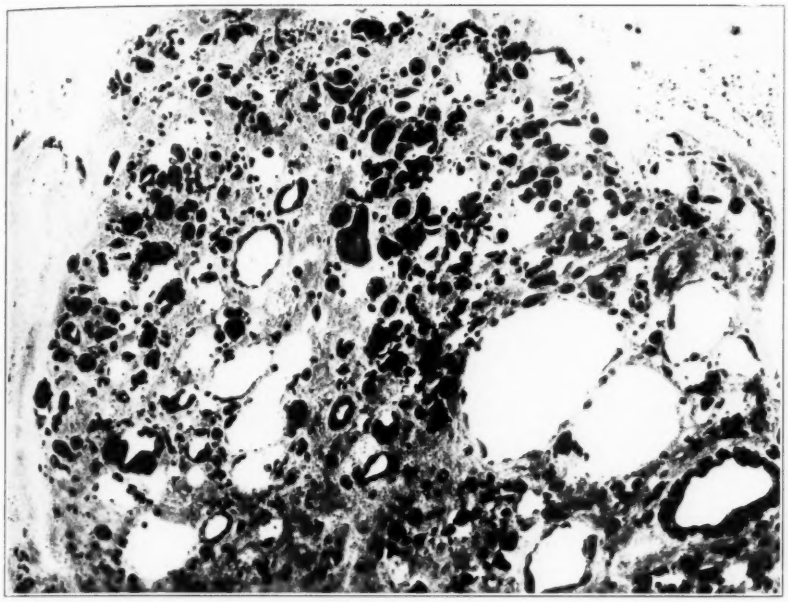
## DESCRIPTION OF PLATE

### PLATE 48

FIG. 1. Scharlach R stain showing dilated sinuses filled with lipoid material. The contents have fallen out of a few of the larger spaces during staining. × 15.

FIG. 2. Hematoxylin and eosin stain of a lymph node showing proliferation of reticulum cells and formation of giant cells. × 250.



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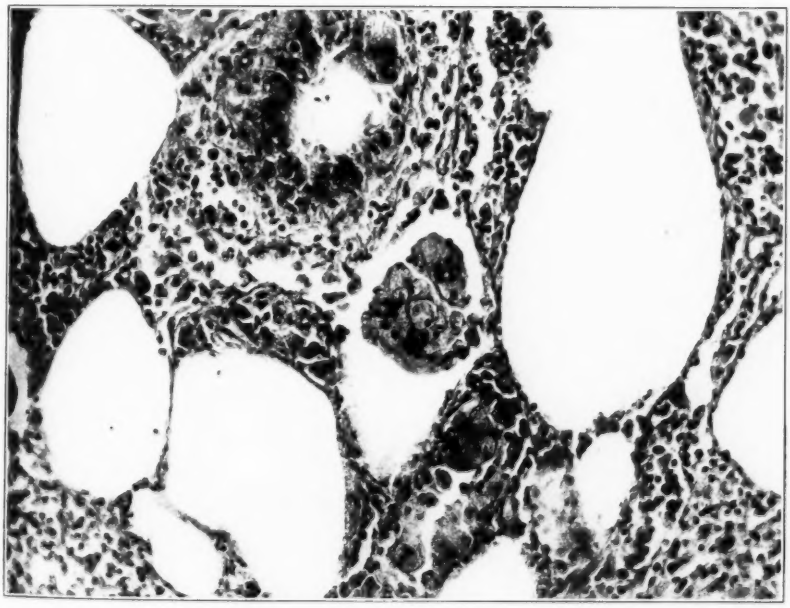

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Hill Mesenteric Chyladenectasis

# DIFFUSE PARIETAL ENDOCARDIAL SCLEROSIS *

## Review of the Literature and Report of Two Cases

Wilfrid J. Comeau, M.D. †

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Acute and chronic valvular endocarditis have received considerable attention in the literature, but there are only a few reports of diffuse parietal endocardial sclerosis to be found. Moreover, most of the reports are somewhat confusing since there are included discussions of endocardial pockets (Zahn), which as isolated findings are to be differentiated, at least macroscopically, from the striking picture of the diffuse mural endocarditis under discussion. It is the purpose of this report to review the literature and to present 2 cases in which unusual pathological changes were found in the myocardium.

### Review of Literature

To clarify the ensuing discussion it seems wise to describe first the architecture of the normal parietal endocardium. Nagayo,[1] after a thorough histological study, concluded that it consisted of five layers. These are, from the surface toward the myocardium, in the following order: (1) endothelial and subendothelial layer; (2) inner connective tissue layer; (3) elastic lamina; (4) smooth muscle layer; and (5) outer vascular connective tissue layer with the fibers of the Purkinje system.

It is evident from the variety of opinions expressed in the literature that there is no established etiological basis for this type of endocarditis. There is, however, a certain amount of agreement that the endocardial fibrosis in a group of these cases is functional in nature and is characterized histologically by the laying down of elastic fibers in a more or less orderly fashion, parallel to the surface, in the inner connective tissue layer of the endocardium.

Böger [2] believes that this group is characterized macroscopically, with only rare exceptions, by a diffuse, uniform grayish white thickening of the parietal endocardium, particularly in the left

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ventricle. Using a teleological argument, he concluded that a compensatory response of the parietal endocardium to prolonged dilatation is the cause of the sclerosis in this group. Hertel [3] makes no gross differentiation and from her material decided that the functional type was a result of prolonged hypertension or chronic myocardial disease. Karsner,[4] writing on sclerosis of the mural endocardium, particularly near the aortic orifice, believes that the fibrosis is essentially the same as intimal sclerosis with a possible inflammatory component. Nagayo [1] and Herxheimer [5] regard the parietal fibrosis seen in aged individuals, and particularly those with aortic valvular incompetence, to be the result of a chronic mechanical irritation by friction of the blood stream.

In the non-functional group the cause of the endocardial fibrosis has been given as contact or direct extension of the inflammation from valvular disease (Böger, Hertel, Dewitzky[6]), thrombosis of the vessels in the region of the endocardium (Nagayo, Hertel), extension from a myocarditis (Hertel, Dewitzky), and primary inflammation of the mural endocardium (Hertel, Dewitzky). Aschoff [7] believes that the majority of the cases without valvular disease are the result either of a parietal thromboendocarditis or of an atypical form of endocardial inflammation.

Mention must be made of the circumscribed elevated plaques that were a striking feature of the 2 cases reported here. One gathers from the literature that these plaques can occur with or without diffuse fibrosis and may or may not show pocket formation. It is difficult to decide from the reports and discussions whether or not isolated plaques are to be considered an entirely different pathological entity from those associated with more or less diffuse mural fibrosis. There is one point which may be of differential importance and that is that some of these plaques are described as light yellow rather than grayish white. Böger believes that plaque formation is a differential feature and commonly inflammatory, but rarely functional in origin. Herxheimer, on the other hand, concludes these plaques are of a functional nature. Dewitzky, histologically, differentiates two general groups, functional and inflammatory; the latter either primary on the endocardium or secondary by extension from valvular or myocardial disease.

The impression obtained from the literature is that two general groups of parietal endocardial fibrosis exist; one functional, the

other inflammatory. There is, however, little agreement as to the group in which many of the reported cases belong. This is due partly to the variety of histological and gross findings observed in this condition and partly to the different standards that are taken for classification.

## Material

In Case 1 it was possible to examine only the heart. Other information was obtained from the routine protocol. Blocks of tissue including endocardium and myocardium were taken from the larger fibrous plaques and other portions of both ventricles. Sections were also made from both auricles and the aorta. In Case 2 the heart was desired as a museum specimen and material was taken from the cut surfaces of both ventricles. The larger plaques were not investigated but smaller plaques were included in the material studied. The auricles and aorta, as well as the nodules on the mitral valve, were investigated. A large number of sections were made from each block. Hematoxylin and eosin, hematoxylin and Sudan III, and a combination of Weigert's elastic and Van Gieson's connective tissue stains were used.

## Case Reports

Case 1. *Clinical Summary:* The patient was a 41 year old male who had always been in good health and active athletically. His illness began a year before death and was characterized by a gradually increasing number of fainting spells which developed into epileptiform attacks, and the progressive development of non-valvular cardiac failure with periods of bradycardia. There was no past history or clinical evidence of infection. The Wassermann reaction was negative. Repeated electrocardiogram tracings showed either a right or left bundle-branch block or complete heart block. The type of heart disease could not be determined after thorough clinical investigation.

*Clinical Diagnosis:* Myocarditis with Adams-Stokes' syndrome.

### *Autopsy*

A complete postmortem examination was performed. Pathological changes, other than in the heart, consisted of congestive changes in the lungs, liver, spleen and kidneys. There was a considerable degree of edema of the brain.

### *Macroscopic Findings in the Heart*

The heart weighed 385 gm. There was a high degree of dilatation of the right and left ventricles. The ventricular muscle meas-

ured 1.5 cm. on the left and 0.8 cm. on the right. There was a striking, irregularly diffuse, grayish white discoloration of the parietal endocardium of both ventricles, which was much more extensive on the left.

Scattered throughout the mural endocardium were elevated and more or less circumscribed plaques which in a number of places were confluent. There was one large confluent plaque on the septum below the right coronary aortic valve and a smaller plaque just below the septum fibrosum. There was a large plaque situated on the right septal endocardium just below the pulmonary orifice. The surface of the papillary muscles was peppered with smaller plaques and areas of diffuse endocardial thickening. The aorta showed no evidence of arteriosclerosis or syphilis and both coronaries were patent and smooth along their entire visible course.

*Histological Examination*

The striking change in the endocardium was found in the inner connective tissue layer. This was widened, particularly in the region of the plaques, and the elastic element played the dominant rôle in this change. In the areas of more or less diffuse endocardial thickening these elastic fibers were long and laid down in an orderly fashion parallel to the surface. As the plaques were formed they became shorter and thicker and, although intermeshing with each other to form a dense mass, maintained a general orderly arrangement. A thin outer zone of this layer did not seem to take part in this process and a narrow red band of connective tissue could be seen clearly just above the elastic lamina in the Weigert-Van Gieson preparations. Blood channels were scattered throughout this abnormal inner connective tissue layer. The elastic lamina was widened and from the outer border there was an outgrowth of elastic fibers. The architecture of the outer two layers was completely lost so that below the elastic lamina there was an irregular outgrowth of connective tissue which enmeshed degenerated muscle fibers, and in many places the conduction system was involved in this process and was badly damaged. Lymphocytes, scattered or in groups, were found in this outer layer and infiltrated the Purkinje system in many sections.

These lymphocytes, scattered, but mainly in miliary to submiliary sized foci, were found throughout the myocardium. Some of these

foci were enmeshed in a network of young fibroblasts. In addition there were foci similar to the latter which contained two or three giant cells. These giant cells were not of the Langhans type but resembled the foreign body form. They were elliptical in shape with an eosinophilic cytoplasm and many basophilic nuclei scattered around the periphery. These giant cell foci were for the most part perivascular, particularly around the medium sized venules. In some sections strands of connective tissue from the perivascular area were seen infiltrating into these foci.

In addition to a fatty degeneration of muscle fibers directly beneath the endocardium, degenerated fibers were occasionally found within the myocardium in nests of connective tissue and occasionally there were definite old myocardial scars. All of these scars were small. There was a general increase of the myocardial connective tissue, especially noticeable in the perivascular region. Only minimal arteriosclerotic changes were revealed by the fat stain.

Scattered foci of lymphocytes were present in the auricular myocardium but no giant cells or abnormal fibrosis were noted. The auricular endocardium was thickened, particularly on the left, with a definite increase in the elastic element. The aorta was normal in all of its layers.

### *Comment*

The damage found in the conduction system seems sufficient to explain the clinical evidence of heart block. The findings do not, however, explain the changing nature of the block. The factors responsible for such fluctuations are discussed in a forthcoming publication.[8]

It is such cases of endocardial sclerosis as this, occurring in relatively young individuals without evidence of arteriosclerosis or valvular disease, that have been infrequently described and studied. In this case the myocardium was the site of pronounced changes. These changes were characterized by an infiltration of lymphocytes which in foci, particularly perivenous, contained giant cells and young fibroblasts. There were no Aschoff bodies or other evidence of a rheumatic infection. In view of the giant cell foci tuberculosis or syphilis appears to be the outstanding possibility. The lack of the epithelioid element, the type of giant cell, the absence of tuberculosis elsewhere in the body, in addition to the fact that these foci in no

way resembled the architecture of the tubercle with its caseous center, definitely eliminate tuberculosis in its classical form. Against syphilis is the absence of a syphilitic mesaortitis, the non-gummatous character of the foci, and the negative Wassermann reaction. Consequently an isolated syphilitic myocarditis seems very unlikely.

Certainly it is reasonable to consider the various histopathological findings as different stages of the same process with fibrosis, particularly perivascular, as the end result. The picture as a whole definitely suggests a chronic infectious process, the nature of which cannot be determined from either the clinical or the pathological data.

In view of the findings the endocardial fibrosis must be considered secondary to the myocardial process. The fact that the endocardial change is mainly in the inner connective tissue layer, that the elastic fibers are more or less laid down orderly and, apparently, over a normal band of connective tissue, and also that the elastic lamina is unbroken, favor the conclusion that the fibrosis is a change compensatory for a subendocardial weakness. The changes in the lower layers are considered for the most part to be due to the myocardial process with pressure from the overlying fibrosis playing a possible rôle.

CASE 2. *Clinical Summary:* The patient was a 47 year old female who had always been well except for "double pneumonia" following a middle ear infection in 1926. Her final illness dated from 1933 and covered a period of 3 years, during which time she was more or less under continual hospital observation. The patient's original complaints were chronic fatigue, nervousness, diarrhea and loss of 20 pounds in weight.

Her illness was characterized by a continual elevation of the basal metabolic rate which resisted all medical treatment and which usually ranged between +40 and +50, although records as high as +80 and +100 were obtained on several occasions. In addition there were a hypotension averaging 90/50 mm. Hg., a fading brownish pigmentation of the skin, an inconstant prominence of the eyeballs, persistent diarrhea (3 to 5 movements daily), no gain in weight, non-valvular cardiac enlargement, and slowly progressive cardiac failure with attacks of pulmonary edema. There were no signs of infection. The urinary and blood findings, including the Wassermann reaction, showed no abnormality, excepting a moderate hypochromic anemia. The electrocardiogram tracings showed evidence of myocarditis and were suggestive of coronary infarction.

*Clinical Diagnoses:* Hyperthyroidism, myocardial failure and adrenal insufficiency.

### *Autopsy*

A complete postmortem examination was performed. The findings, exclusive of the heart, were colloid goiter without enlargement

or toxic changes, and congestion of the lungs, liver, spleen and kidneys. The adrenals and hypophysis showed no unusual histological changes. Foci of lymphocytes with foreign body giant cells were found in the lung alveoli and in the periportal connective tissue of the liver.

### *Macroscopic Findings in the Heart*

The heart weighed 420 gm. There was a high grade chronic dilatation of the left ventricle which was aneurysmal at the apex. There was an additional dilatation of the left auricle and a dilatation and hypertrophy of the right ventricle. The ventricular muscle measured 1.6 cm. on the left, and 0.9 cm. on the right. The valves were normal with the exception of the mitral. Along the free border of both leaflets of this valve pin-head sized fibrous nodules were found and the border of the valve was slightly thickened generally. The mitral orifice measured 10 cm.

The left parietal ventricular endocardium showed a striking, diffuse grayish white discoloration with plaque formation. The larger plaques were situated mainly in the posterior portion of the septum beneath the anterior leaflet of the mitral valve. There was a small plaque on the septum below the right coronary aortic valve and smaller scattered plaques were sprinkled over the papillary muscles. The right ventricular endocardium was minimally affected. There were a few small areas of grayish white thickening on the mural endocardium just below the attachment of the auricular leaflet of the tricuspid valve and on the papillary muscles below the pulmonic orifice. The left auricular endocardium seemed somewhat thickened. The aorta and coronary vessels showed no gross evidence of arteriosclerosis.

### *Histological Examination*

The myocardium of the left ventricle was riddled with small fibrous scars, a few of which were definitely perivascular. There was also an infrequent scattered collection of lymphocytes. Most of the blood vessels were distended and filled with blood, and fresh extravasated blood was seen in the connective tissue and between the muscle fibers. The right ventricular myocardium showed infrequent groups of three or four small scars. No Aschoff bodies were noted nor was arteriosclerosis of the smaller vessels found.

As in the previous case the inner connective tissue layer of the endocardium showed striking changes. The histopathological findings in this layer were comparable with those found in Case 1 except that the outer red band of connective tissue was not seen in the Weigert-Van Gieson preparations. The elastic lamina was widened but unbroken. The smooth muscle layer was not recognized as such so that under the elastic lamina there existed a layer of increased connective tissue which fused, for the most part, with scarred areas under the endocardium or invaded patches of muscle fibers, some of which were degenerating.

The auricular myocardium showed no abnormal changes while the auricular endocardium showed slight thickening. The nodules on the mitral valve were composed of scar tissue with a slight elastic element, but no Aschoff bodies were found. The aorta was normal.

*Comment*

The pathological changes in this case are sufficiently clear to explain the cardiovascular aspects of the clinical picture. A thorough pathological examination failed, however, to reveal a basis for the metabolic features of the patient's illness. No explanation can be offered for the giant cell foci found in the lungs and liver. The fact that the patient in the past had worked as a silk weaver might, on the basis of inhalation of silk particles, have some bearing on their formation.

In regard to the myocardium it is unlikely that the scarring was due to arteriosclerosis. The small size of the scars, their dissemination through both the left and the right ventricular myocardium, and the absence of arteriosclerotic changes macroscopically and microscopically, leave only extremely remote possibilities based on an arteriosclerotic genesis. No Aschoff bodies were found and, in view of Grant's study,[9] there is little reason to assume that the changes in the mitral valve were rheumatic in origin. The myocardial scarring, however, could in no way be considered as a residuum of a rheumatic infection. The hemorrhage is regarded as a postmortem change.

The scarring was definitely an old process. Its small disseminated character and the history of a middle ear infection complicated by pneumonia strongly suggest the possibility that the histological picture is the end result of an embolic myocarditis. As for the lympho-

cytic infiltration, it is impossible to say whether it is a residuum of an infection or the result of an excessive effort of a badly damaged myocardium. Again, as in Case 1, no positive conclusions can be drawn but from the available evidence an infectious etiology for the myocardial lesion is regarded as the most probable.

The endocardial changes in this case closely resembled those found in the previous case. The main change was in the inner connective tissue layer of the endocardium and consisted of the laying down of elastic fibers in a more or less orderly fashion parallel to the surface. The elastic lamina was intact and definitely reinforced by new fibers. No changes were noted beneath the elastic lamina that could not be attributed to the myocardial process or to pressure. The possibility of the diffuse endocardial fibrosis resulting in any way from the process on the mitral leaflets is remote. The evidence of prolonged severe myocardial damage and the character of the histological changes in the endocardium again suggest a compensatory reaction on the part of the endocardium.

## Discussion

In reviewing these 2 cases it can be said from the pathological evidence, as interpreted above, that in a group of cases of parietal endocardial sclerosis the changes may be considered as compensatory for a prolonged and severe subendocardial weakness. Although it so happened that in the cases reported here the myocardial process was most likely infectious in origin, it is felt that the same endocardial changes can occur from any process resulting in prolonged and severe myocardial debility. In these cases the change may be considered functional, but functional only in the well recognized physiological sense that every damaged organ protects itself as efficaciously as possible in order to survive. Thus, the functional change is not primary but secondary to an organic dysfunction of the myocardium, whatever its nature may be and whether it is histologically recognizable or not. In the present state of our knowledge it would only be purely hypothetical to discuss the possible factors that stimulate this protective response of the endocardium.

The plaque formation is interesting and always a striking feature of the macroscopic picture when it exists. The cases reported here do not allow a discussion of the inflammatory etiology of these plaques. That such may be the case, particularly in more or less

isolated plaques, seems quite probable. In the 2 cases presented here the plaque formation must be considered part of the general process, developing without inflammation of the endocardium. Consesequently it is felt that the formation of fibrous plaques cannot be considered as a differential feature between functional and inflammatory endocardial sclerosis, as Böger suggests. No clue was obtained in this study as to why this compensatory response manifests itself in some cases by plaque formation. It is possible that this occurs over a particularly severely damaged subendocardial area.

## Summary

1. Two cases of diffuse parietal endocardial sclerosis with plaque formation occurring in middle-aged individuals without arteriosclerosis are presented. In 1 case there was no evidence of valvular disease; in the other there was a slight degree of old rheumatic endocarditis of the mitral valve.

2. In each case evidence of prolonged and severe myocardial damage was present. In Case 1 the histopathological findings were unusual, being characterized by diffuse and focal lymphocytic infiltrations a few of which, particularly the perivenous, contained giant cells of the foreign body type. Fibrosis, especially the perivascular type, appeared to be the end stage of the process. In Case 2 diffusely disseminated small scars were found in the myocardium of both ventricles, particularly extensive in the left. Both cases are regarded as infectious in origin; the former, a chronic infection of unknown nature; the latter, the end stage of a previous metastatic myocarditis.

3. From the pathological findings it is concluded that in a group of cases of diffuse parietal endocardial sclerosis with plaque formation there is a compensatory protective change in the endocardium secondary to an organic subendocardial weakness. The organic nature of this weakness is not considered specific but may result from any process directly or indirectly affecting the myocardium which causes prolonged and severe weakness of the ventricular musculature.

4. It is concluded that parietal fibrous plaques may develop on such a basis and consequently cannot be used to differentiate between infectious and functional endocardial sclerosis.

NOTE: I wish to express my appreciation for the invaluable help given me in this study by Professor Aschoff. I also wish to thank Dr. Leo Müller of the Stadt Krankenhaus, Baden-Baden, for the opportunity of studying the heart in Case 1.

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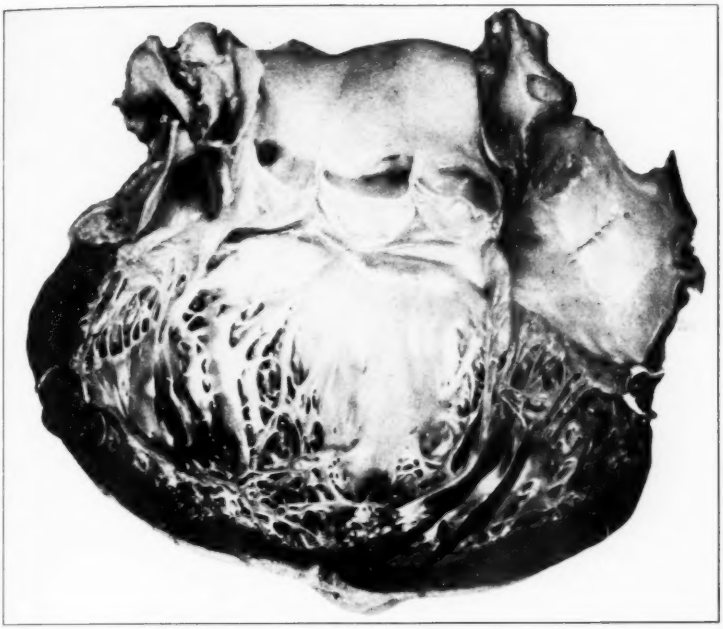
## DESCRIPTION OF PLATES

### PLATE 49

FIG. 1. Case 1. Left ventricle.

FIG. 2. Case 1. Right ventricle.



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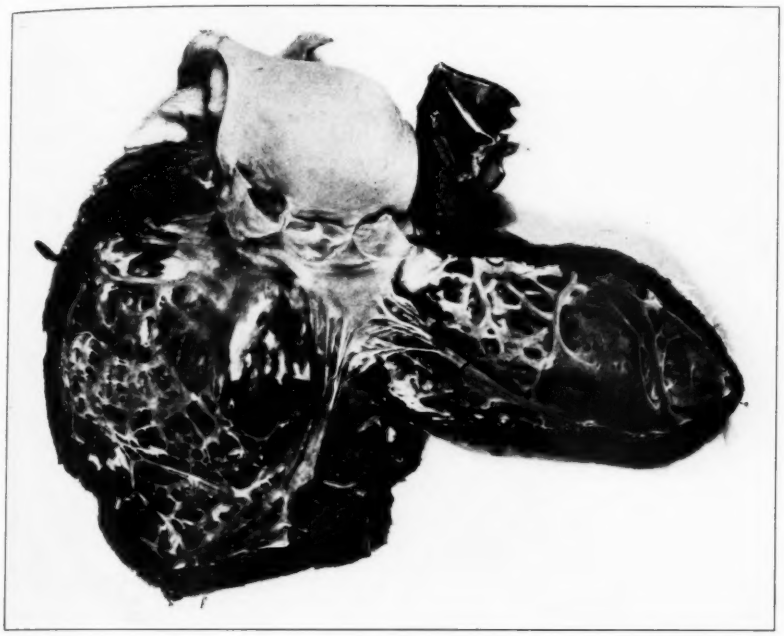
Comeau Diffuse Parietal Endocardial Sclerosis

PLATE 50

FIG. 3. Case 2. Left ventricle.

FIG. 4. Case 1. Focus of lymphocytes, giant cells and fibroblasts adjacent to a venule. Hematoxylin-eosin stain.



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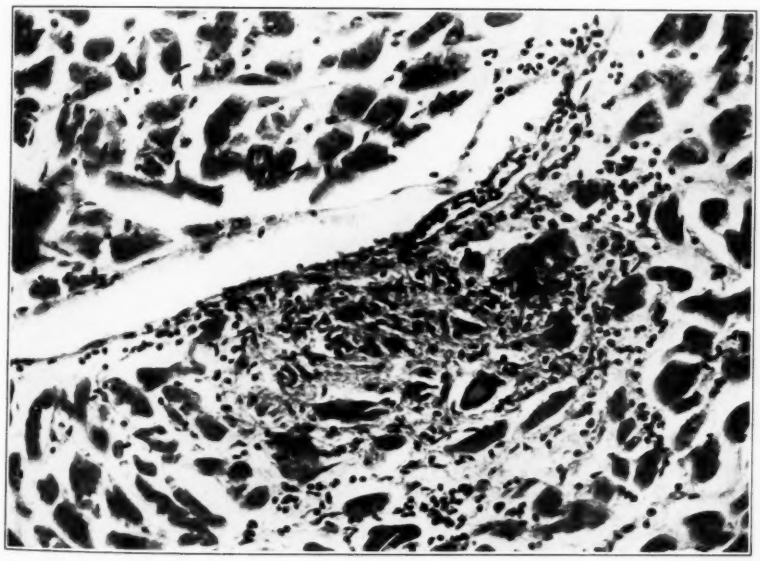

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Comeau

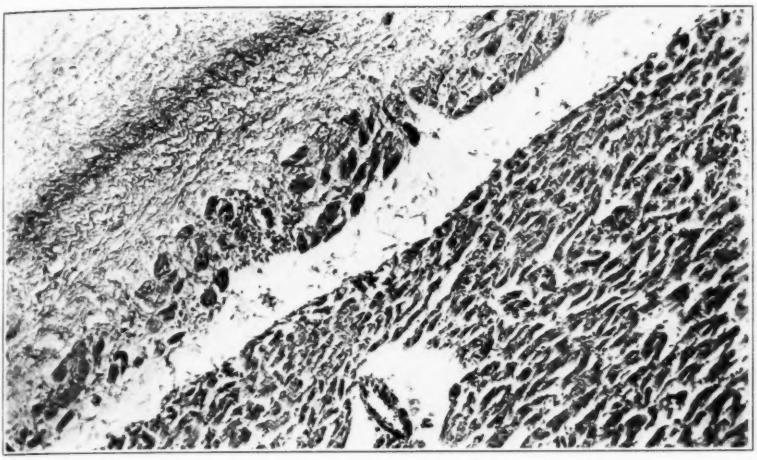
Diffuse Parietal Endocardial Sclerosis

PLATE 51

FIG. 5. Case 1. Showing a portion of the left branch of the conduction system invaded by fibroblasts and lymphocytes. Hematoxylin-eosin stain.

FIG. 6. Case 1. Endocardium. The base of connective tissue above the elastic lamina can be plainly seen. Degenerated muscle fibers enmeshed in connective tissue are seen below the elastic lamina. Weigert's elastic tissue and Van Gieson's connective tissue stain.

FIG. 7. Case 2. Endocardium. The parallel arrangement of the elastic fibers can be seen on the right. On the left they form a denser meshwork. Weigert's elastic tissue and Van Gieson's connective tissue stain.



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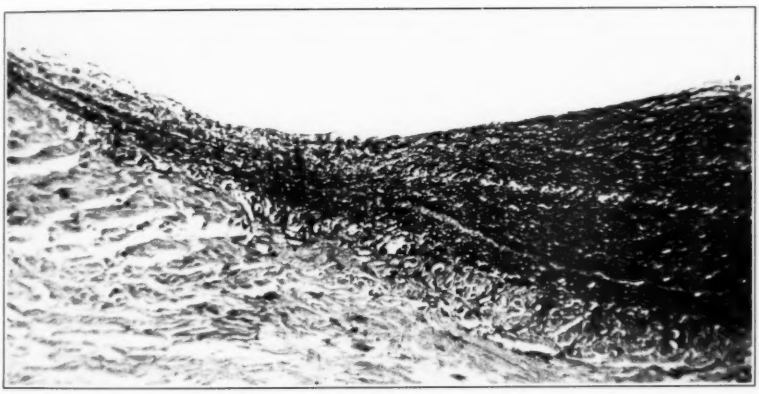

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Comeau Diffuse Parietal Endocardial Sclerosis

# COMBINED INFANTILE AND ADULT COARCTATION OF AORTA WITH COINCIDENT OCCLUSION OF VENA CAVA SUPERIOR*

## Report of a Case

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Coarctation of the aorta is not altogether a rare lesion. Dr. Maude Abbott [1] reported and reviewed 200 collected cases with autopsy in 1928. Mention was made in this article of 5 additional unpublished cases with autopsy. The relative infrequency of the condition may in part be accounted for by the failure of observers to record their cases. We have seen several prior to the case reported here and have reported none of them.

Two types of coarctation of the aorta are recognized: (1) the adult form in which the stenosis occurs at the level of the insertion of the ductus arteriosus or slightly proximal to this point; and (2) the infantile variety consisting merely of a narrowing of the descending portion of the aortic arch at the level of the inferior border of the origin of the left subclavian artery. As a rule one or the other type alone is present.

The etiological factors responsible for coarctation and the complications resulting from it have been adequately discussed by Abbott [1] and need not be mentioned here. It will be sufficient to mention at this time only that the most common findings associated with aortic coarctation are bicuspid aortic valve, with or without a superimposed bacterial endocarditis, subaortic stenosis, dilatation and hypertrophy of the heart, dilatation of the ascending aorta, arterial collateral circulation, anomalous origin of the arteries of the arch, hypoplasia of the aorta, persistent left superior vena cava, defects of the aortic septum, obliterative pericarditis and pleuritis, dissecting aneurysm of the aorta and aneurysmal dilatation of the cerebral vessels.

The above group of complications is usually associated with the adult form. Accompanying the infantile variety may be found more

* Received for publication July 17, 1936.

TABLE I

*Analysis of 75 Cases of Coarctation of Aorta*

(1928–1935)

| Author and year | Age and sex | Infantile type | Adult type | Collateral circulation | Associated anomalies | Secondary findings and other pathology | Cause of death | Clinical data |
|---|---|---|---|---|---|---|---|---|
| Green, F. H. K.[2] 1928 | Male 21 yrs. | Mod. stenosis at isthmus admitting lead pencil, just distal lt. subclavian art.* D. A. oblit. | | No mention | Bicuspid aortic valve | Mitotic congenital cerebral aneurysms | Sudden death; rupture congenital aneurysm basilar cerebral artery | |
| Jordan, F. L. J., and ter Harr, F. T.[3] 1928 | Not given | | Atresia from origin lt. subclavian art. to oblit. D. A. | Increased | No mention | Hypertrophy & dilatation heart; fusiform aneurysm aortic arch above coarctation | Cardiac death | Pulsating carotids; capillary pulse; aortic insufficiency; Wassermann negative; loud interscapular murmur |
| Anderson, R. G.[4] 1928 | Male 44 yrs. | Mod. stenosis aortic isthmus lumen 1.27 cm. diam. D. A. oblit. | | Increased | Malignant endocarditis | | Cardiac death; malignant endocarditis | |
| Rösler, H.[5] 1928 | Not given | Stenosis aortic isthmus. D. A. oblit. | | No mention | No mention | | | |
| Poynton, F. J., and Sheldon, W. P. H.[6] 1928 | Male 8 yrs. | Mod. stenosis 1.1 cm. below lt. subclavian art. lumen narrowed to half original diam. D. A. oblit. | | No mention | Bicuspid aortic valve; ulcerative aortitis beyond coarctation | Kidneys & spleen enlarged & contain recent infarcts | Positive hemolytic streptococcus blood culture | |
| Houck, G. H.[7] 1929 Case 1 | Male 49 yrs. | | Coarctation | | | | Pneumonia | |
| Case 2 | Male 29 yrs. | | Coarctation | | | | Miliary tuberculosis | |
| Weber, F. P., and Knop, F.[8] 1929 | Male 54 yrs. | | Extreme stenosis aorta 1.3 cm. distal origin lt. subclavian art. at site insertion oblit. D. A. | Increased | Bicuspid aortic valve | Hypertrophy & dilatation heart; chronic oblit. pericarditis; chronic oblit. pleurisy; calcareous & verrucose cusps; calcification aortic ring | Lobar pneumonia | Ice block carrier |
| Bode, O. B., and Knop, F.[9] 1929 Case 1 | Male 54 yrs. | | Coarctation at oblit. D. A. | Increased | | | Cardiac death | |
| Case 2 | Male 40 yrs. | | Coarctation at oblit. D. A. | No mention | | Hypertrophy left ventricle mainly | Cardiac death | Laborer — heavy work |
| Andreesen, R.[10] 1929 Case 1 | Male 47 yrs. | | Stenosis aorta site oblit. D. A. lumen 3 mm. diam. | Increased | Dissecting aneurysm base ascending aorta | Hypertrophy & dilatation heart; coronary arteriosclerosis; slight dilatation upper portion descending aorta; mesaortitis luica | Hemopericardium, rupture walls aneurysm | |
| Case 2 | Male | | Mod. stenosis aorta | Moderate | | Hypertrophy & dilatation heart | Drowning | |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 1930 Case 1 | 47 yrs. | | 3 mm. diam. | | aorta | slight dilatation upper portion descending aorta; meso-aortitis luica | aneurysm | |
| Case 2 | Male 18 yrs. | | Mod. stenosis aorta after origin lt. subclavian & just below oblit. D. A. lumen size lead pencil | Moderate | | Hypertrophy & dilatation heart | Drowning | |
| Case 3 | Male 47 yrs. | Shelf-like stenosis directly beyond origin lt. subclavian art. | Extreme stenosis site oblit. D. A. lumen admits fine probe | Moderate | | Fusiform aneurysmal dilatation descending aorta just beyond 2nd stenosis | Myocardial degeneration | |
| Case 4 | Male 48 yrs. | | Mod. stenosis just beyond site oblit. D. A. & 1.5 cm. beyond single vessel arising from arch | Increased | Bicuspid aortic valve; anomalous single vessel leaves aortic arch | Hypertrophy & dilatation heart; saccular aneurysmal dilatation ascending aorta; atheroma aorta | Hanging | |
| Thomson, A. P., and Lamb, F. W. M.[11] 1929 | Female 5 yrs. | | Extreme stenosis ½" below origin lt. subclavian art. at site oblit. D. A. | No mention | | Hypertrophy & dilatation heart — mostly on lt. side; dilatation ascending aorta; congenital abnormality lt. kidney; hypoplasia rt. kidney | Cardiac death | |
| Sala, A. M., and Nachamie, I.[12] 1929 | Male 6 yrs. | | Extreme stenosis site insertion oblit. D. A. | None | | Hypertrophy & dilatation lt. ventricle heart | Bilateral confluent bronchopneumonia; localized necrosis of lt. bronchus | |
| Fray, W. W.[13] 1930 | Male 57 yrs. | | Complete atresia site oblit. D. A. | Increased | Subaortic endocarditis with stenosis; ulcerative aortic endocarditis with extension into tricuspid ring | Hypertrophy & dilatation heart, mostly lt. ventricle; hypoplasia abdominal aorta; sl. dilatation ascending aorta | Pulmonary thrombosis; ulcerative aortic endocarditis | Good health until P. I. Onset with fever, dyspnea, weakness. Pulsating neck veins in thoracic axillary & scapular artery. Pos. bl. cult. *Str. viridans*. X-ray shows absent aortic knob & rib erosion |
| Terbrüggen, A.[14] 1931 | Not given | | Atresia aorta site insertion oblit. D. A. | Moderate | | Hypertrophy heart | Cardiac death | |
| Ernstene, A. C., and Robins, S. A.[15] 1931 | Male 47 yrs. | | Atresia short distance below insertion oblit .D .A., stenosis 1.5 cm. on each side | Increased | | Rib erosion limited to post. half ribs & inferior borders | Cerebral hemorrhage | Rib erosion shown by X-ray; cramps, weakness leg 5 yrs. Dyspnea, palpitation, pulsating neck vessels; B. P. upper extremity 164/90, lower extremity 88/76; pulse tracings showed lag of femoral over radial |

* D.A. = ductus arteriosus

TABLE I—*continued*

| Author and year | Age and sex | Infantile type | Adult type | Collateral circulation | Associated anomalies | Secondary findings and other pathology | Cause of death | Clinical data |
|---|---|---|---|---|---|---|---|---|
| Costa, A.[16] 1931 | Female 54 yrs. | | Atresia aorta site insertion oblit. D. A. | Increased | | Aneurysmal dilatation 1st portion ascend ng aorta; hypertrophy, dilatation heart, mostly lt. side; 1 cm. from origin innominate art. showed aneurysma dilatation 8 cm. cir. with thrombotic deposits; 2 cm. caudal to atresia saccular aneurysmal dilatation post. rt. wall aorta; softened white rt. cerebral hemisphere | Cerebral embolism | Domestic servant |
| Ulrich, H. L.[17] 1932 | Male 23 yrs. | | Stenosis aorta site insertion patent D. A. | Increased | | Hypoplasia descending aorta beyond coarctation, dilatation proximally; hypertrophy, dilatation heart; slight coronary arteriosclerosis; splenomegaly; portal cirrhosis; chr. pass. cong. viscera; ascites; fatty degen. liver | Cardiac death | |
| Lemon, W. S.[18] 1931 | Male 22 yrs. | | Stenosis where large patent D. A. entered aorta | No mention | Bicuspid aortic valve | Hypertrophy, dilatation heart; arteriosclerosis aorta & coronary artery; neurofibroma mediastinum | | Pulsating vessels neck & thorax |
| Hein, G. E.[19] 1931 Case 1 | Male 11 days | Atresia aorta proximal to widely patent D. A. | | No mention | Defect 0.5 cm. diam. in interventricular septum | Dilatation, hypertrophy rt. heart; dilatation ascending aorta; foramen ovale anatomically patent | | |
| Case 2 | Male 32 yrs. | Narrowing aorta at isthmus | | | | Autopsy showed syphilis | | |
| Case 3 | Male 60 yrs. | Narrowing aorta at isthmus | | | Aortic stenosis, calcification | Hypertrophy & dilatation heart; arteriosclerosis | | |
| Abbott, M. E.[20] 1932 | Female 34 yrs. | Narrowing aorta at isthmus | Extreme stenosis level insertion oblit. D. A. lumen 1.8 cm. cir. | Moderate | Bicuspid aortic valve (probably congen.) with calcified raphe; productive sclerosing aortic endocarditis | Hypertrophy & dilatation heart; dilatation ascending aorta; tricuspid & mitral stenosis | | |
| Laubry, Ch., Routier, D., and Van Bogaert, A.[21] 1932 | Male 42 yrs. | Stenosis aortic isthmus just below origin lt. subclavian art. | | No mention | | | Pulmonary gangrene | B. P. 200/130 both arms; unable to record legs; pulsation neck vessels; previous pneumonia |
| Narr, F. C., and Wells, A. H.[22] 1933 | Male 29 yrs. | Stenosis level of origin lt. subclavian art. D. A. oblit. at pulmonary end | | No mention | Dissecting aneurysm ascending aorta | Hypertrophy, dilatation heart; arteriosclerosis coronary artery; fenestrated aortic valve | Hemopericardium. Rupture of wall of dissecting aneurysm 1 cm. from the base of aorta | |

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|---|---|---|---|---|---|---|---|---|
| East, T.[32] 1932 | Female 46 yrs. | | Stenosis just below entrance oblit. D. A. | Increased | Rt. subclavian takes origin from lt. side aortic arch | Sl. hypertrophy lt. ventricle; sl. arteriosclerosis; infantilism bicornate uterus; osteitis deformans rt. tibia & skull | Fracture of skull | |
| Strong, G. F.[34] 1932 Case 1 | Male 19 yrs. | | Atresia at & below insertion oblit. D. A. | Moderate | Bicuspid aortic valve due to congenital fusion coronary cusps; rheumatic valvular endocarditis, subaortic stenosis; anomalous vessels | Hypertrophy & dilatation heart; dilatation descending aorta below constriction; hypoplasia descending aorta | Hemopericardium. Rupture of a dilated and thinned ascending aorta | |
| Case 2 | Male 39 yrs. | | Stenosis 2 cm. inferior to origin lt. subclavian at site of insertion oblit. D. A. | Increased | Slight fusion coronary cusps | Enlarged heart; arteriosclerosis ascending aorta; anasarca; infarcts rt. lung | Cardiac death | Rib erosion shown by X-ray |
| Case 3 | Female 12 yrs. | | Stenosis level of insertion D. A., oblit. pulmonic side | None | | Sl. hypertrophy & dilatation heart | Chronic nephritis, bilateral hydronephrosis, terminal bronchopneumonia | |
| Read, W. T. Jr., and Krumbhaar, E. B.[36] 1932 | Male 35 yrs. | | Stenosis just below oblit. D. A. lumen 1.4 cm. diam. | Increased | Bicuspid aortic valve; anterior leaflet site of calcareous dep. | Hypertrophy, dilatation heart; hypoplasia aorta; coronary arteriosclerosis with myofibrosis; arteriolonephropathy | Cardiac death | B. P. 200 mm. Hg.; cyanotic & unconscious; fluid pouring from mouth |
| Carnett, J. B., and Howell, J. C.[26] 1932 | Male 75 yrs. | | Stenosis aorta site oblit. D. A. | Increased | | Dilatation ascending aorta; hypoplasia aorta distal to coarctation; mod. hypertrophy & dilatation aorta | Terminal bronchopneumonia, pulmonary edema | Wass. Neg.; dyspnea. B. P. rt. arm 180/80, lt. arm 160/80; rt. leg 140/96, lt. leg 100/30; X-rays show notching of ribs |
| Schleckat, O.[27] 1933 | Male 44 yrs. | Mod. stenosis aortic isthmus | | | | Hypertrophy & dilatation heart | Terminal bronchopneumonia | Wassermann positive |
| Root, J. H.[28] 1933 | Male 13 days | Stenosis aorta between openings left carotid & lt. subclavian art. lumen 8 mm. cir. D. A. not identified | | No mention | Bicuspid aortic valve, fusion coronary cusps | Hypertrophy & dilatation heart | Cardiac death | |
| Pozzan, A.[29] 1933 | Male 42 yrs. | | Atresia aorta site oblit. D. A. | Increased | Bicuspid aortic valve showing thick calcareous plate involving portion pars membranacea septi; dissecting aneurysm ascending aorta | Hypertrophy & dilatation heart mostly lt. side | Hemopericardium, rupture dissecting aneurysm aorta at base | Robust, athletic type |

TABLE I—*continued*

| Author and year | Age and sex | Infantile type | Adult type | Collateral circulation | Associated anomalies | Secondary findings and other pathology | Cause of death | Clinical data |
|---|---|---|---|---|---|---|---|---|
| Lewis, T.[30] 1933 Case 1 | Male 43 yrs. | | Stenosis aorta immediately beyond insertion oblit. D. A. Lumen a slit 0.5 cm. × 1.5 cm. | Increased | Patches sessile vegetations aortic cusps (organized subacute bacterial endocarditis) commissures fused | Hypertrophy, dilatation heart, mostly lt. side; dilatation ascending aorta; bilateral fibrinous pleurisy, numerous infarcts both lungs; anasarca | Cardiac death | |
| Case 2 | Male 49 yrs. | Aorta narrows beyond left subclavian art. | Atresia immediately beyond insertion oblit. D. A. | Increased | Annular sclerosis aortic cusps | Sl. dilatation ascending aorta; hypertrophy, dilatation heart, mainly left ventricle; coronary arteriosclerosis | Cardiac death | Systolic murmur over chief vessels; no pulsations leg artery; large pulsating vessels neck, interscapular & axillary regions |
| Case 3 | Male 68 yrs. | | Stenosis aorta 2 cm. beyond origin lt. subclavian art. D. A. oblit. Lumen aorta at stenosis 6 × 11 mm. | Moderate | Bicuspid aortic valve, fusion coronary cusps | Hypertrophy & dilatation heart, mainly left ventricle; arteriosclerosis; frontal convolutions brain atrophied; patchy myofibrosis heart; chr. pass. cong. lungs; stones gall bladder | Coronary arteriosclerosis, thrombosis | |
| Giroux, M., and Jobin, J. B.[31] 1933 | Female 20 yrs. | | Atresia aorta at level oblit. D. A. | No mention | Foramen ovale patent | Hypertrophy & dilatation heart; vegetative mitral endocarditis (subacute bacterial); hypoplasia abdominal aorta; interstitial, degenerative myocarditis; ascites; chr. pass. cong. viscera; small thrombus right axillary artery | Cardiac death | B. P. 220/40; dyspnea; peripheral edema |
| Stewart, H. L., and Bellet, S.[32] 1934 | Male 26 yrs | | Stenosis immediately below insertion oblit. D. A. Lumen admits bristle | Increased | Dissecting aneurysm ascending aorta | Hypoplasia descending aorta; no syphilis or arteriosclerosis | Hemopericardium. Ruptured wall dissecting aneurysm at base aorta | |
| Evans, W.[33] 1933 Case 1 | Male 2 wks. | Stenosis aortic arch proximal to origin lt. common carotid. D. A. widely patent, 0.15 cm. | | No mention | Left vertebral artery arises from narrowed portion aortic arch; mitral orifice absent, no communication between lt. ventricle and auricle; patent interventricular septum (0.5 cm.), foramen ovale patent (0.5 cm.) | Rt. heart hypertrophy & dilatation; Meckel's diverticulum | Cardiac death | |
| Case 2 | Male 30 yrs. | Consid. stenosis aorta between orifices lt. common carotid & lt. subclavian art. D. A. oblit. (0.8 cm. cir.) | | No mention | | Hypertrophy & dilatation heart; anasarca; cyanosis; gen. arteriosclerosis | Cardiac death | |
| Case 3 | Female | Stenosis aortic | | No mention | Foramen ovale patent | Hypertrophy & dilatation heart | Bronchopneumonia, purulent bronchitis, | |

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| Case 3 | Female 2 wks. | Stenosis aortic isthmus immediately beyond orifice lt. subclavian art. D. A. patent (0.8 cm. cir.) | | No mention | Foramen ovale patent | Hypertrophy & dilatation heart | Bronchopneumonia, purulent bronchitis, extensive areas collapse | |
| Case 4 | Female 2 days | Stenosis aortic arch & isthmus. D. A. widely patent | | No mention | Foramen ovale patent | Rt. ventricle hypertrophy, dilatation | Heart failure | |
| Case 5 | Male 8 yrs. | | Stenosis aorta opposite center orifice of patent D. A. (0.5 cm. diam.) | No mention | | Rt. heart hypertrophy, lt. heart hypoplasia; hypertrophy pulmonary artery; tuberculosis tracheobronchial lymph nodes; anasarca; cyanosis | Cardiac death | |
| Case 6 | Male 5 days | Stenosis at isthmus just distal to origin lt. subclavian art. D. A. patent (0.15 cm. diam.) | | No mention | Foramen ovale patent (5 by 4 mm.); rt. auricle receives both rt. and lt. vena cava; lt. sup. vena cava enters auricle by way coronary sinus passing behind pulmonary vein | Congenital abnormality jejunum | Intestinal obstruction | |
| Case 7 | Female 1 day | Stenosis proximal to opening widely dilated D. A. (1.5 mm. cir.) Lumen aorta 1.1 cm. cir. | | No mention | Patent foramen ovale (0.2 cm.); lt. vertebral artery arising from arch aorta | Hypertrophy rt. ventricle, auricle | Cardiac death | |
| Case 8 | Female 5 wks. | Stenosis above orifice patent D. A. (0.7 cm. cir.) | | No mention | Foramen ovale patent (0.1 cm.); patent interventricular septum (0.7 by 0.3 cm.) | Hypertrophy & dilatation, right heart | Cardiac death | |
| Case 10 | Male 3 mos. | | Stenosis aorta immediately beyond insertion oblit. D.A | No mention | Foramen ovale patent (0.1 cm.) | Slight dilatation & hypertrophy lt. ventricle; no rib erosion | Cardiac death; bronchopneumonia | |
| Case 11 | Male 16 yrs. | | Stenosis aorta just below 1st pair bronchial arteries. D. A. oblit. Lumen admits fine probe | Moderate | | Hypertrophy lt. ventricle; flecks atheroma in ascending aorta, none in descending | Bronchopneumonia | |
| Case 12 | Male 60 yrs. | | Stenosis site insertion oblit. D. A. | Moderate | Lt. vertebral artery takes origin from aortic arch between lt. common carotid & lt. subclavian art. | Dilatation upper portion descending aorta; carcinoma pylorus; brown atrophy heart; atherosclerosis ascending aorta | Pulmonary embolism; thrombosis venae comites meningeal artery, thrombosis artery lower limbs | No rib erosion |

TABLE I—*continued*

| Author and year | Age and sex | Infantile type | Adult type | Collateral circulation | Associated anomalies | Secondary findings and other pathology | Cause of death | Clinical data |
|---|---|---|---|---|---|---|---|---|
| Case 13 | Female 18 mos. | | Stenosis aorta site insertion oblit. D. A. | No mention | | Thrombosis posterior ½ base superior longitudinal sinus & adjacent 2 cm. both lateral sinuses | Heart failure; bronchopneumonia; foreign body in bronchus | |
| Case 14 | Male 35 yrs. | | Stenosis site oblit. D. A. (admits fine probe) | Increased | Bicuspid aortic valve fusion rt. & lt. anterior cusps; calcification valves | Hypertrophy & dilatation lt. ventricle; acute fibrinous pericarditis; septic erosion small pyogenic saccular aneurysm aorta immediately above aortic commissure; vegetations margins of aneurysm, anasarca; septic spleen; anemic infarct kidneys | Hemopericardium; rupture mycotic aneurysm of aorta | |
| Case 15 | Female 6 yrs. | | Stenosis aortic arch site D. A. & isthmus, pulmonary portion D. A. closed. Aneurysm aortic portion D. A. | No mention | | Sl. rt. heart hypertrophy & dilatation; chr. abscess middle lobe rt. lung with focal empyema; purulent infiltration wall D. A., wall false aneurysm & adjacent pulmonary art.; erosion upper lobe of lt. lung by aneurysm D. A.; false aneurysm wall aorta beneath pericardium & to left of roots great vessels lined by organized blood clot | Hemoptysis, bronchopneumonia; rupture mycotic aneurysm D. A. into lung | |
| Case 16 | Male 23 yrs. | | Stenosis aorta at level insertion oblit. D. A. | Increased | Bicuspid aortic valve, interventricular septum filled with vegetations; consid. destruction common rt. & post. cusps; vegetations spread over aortic cusps; anomalous vessel | Hypertrophy & dilatation of heart; vegetations filling smooth lined aneurysm (1.5 cm. diam.) in ant. part base aortic cusp mitral valve; anasarca, generalized sl. arteriosclerosis | Heart failure; aortic incompetence; subacute bacterial endocarditis | |
| Case 17 | Male 38 yrs. | | Stenosis 2.5 cm. beyond lt. subclavian art. (admits probe) D. A. oblit. | Increased | | Saccular aneurysm anterior wall ascending aorta filled with thrombus & showing partial calcification of wall; dilatation descending thoracic aorta (5.0 cm. cir.); anasarca; sl. atherosclerosis distal to stenosis in aorta | Hemorrhage into pericardial adhesions; rupture aneurysmal wall ascending aorta | |
| Case 19 | Male 32 yrs. | | Atresia aortic arch. D. A. oblit. | Increased | Bicuspid aortic valve. Few recent vegetations slightly thickened aortic valve | Hypertrophy & dilatation of heart; dilatation aortic arch, ascending aorta & commissures; gen. arteriosclerosis | Heart failure; aortic incompetence; acute rheumatic endocarditis | |
| Taylor, E. E.[8] | Female | | Atresia aorta level | Increased | Bicuspid aortic | Gen. arteriosclerosis; aneurysm | Hemopericardium; | "Rheumatic pains" |

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| Taylor, E. F.[34] 1934 | Female 59 yrs. | | Atresia aorta level oblit. D. A. | Increased | Bicuspid aortic valve; calcified, fibrosed, fusion of posterolateral anterior cusp | Gen. arteriosclerosis; aneurysm 1st portion ascending aorta; hypertrophy & dilatation heart | Hemopericardium; rupture aneurysm ascending aorta | "Rheumatic pains" back & legs many years; dyspnea |
| Pierce, W. F.[35] 1934 | Male 25 yrs. | | Atresia aorta 18.2 cm. beyond cusps. D. A. oblit. | No mention | | Empyema scars right pleural cavity | Gangrenous abscess mediastinum communicating with thoracic portion aorta & esophagus | |
| Bargi, L.[36] 1934 | Female 19 yrs. | Spur just below origin lt. subclavian art. producing stenosis | Moderate stenosis aorta site D. A. (1.5 cm. cir.) D. A. oblit. | Increased | Bicuspid aortic valve; fusion of lt. post. & ant. cusps | Saccular aneurysmal dilatation between two levels of stenosis; hypertrophy & dilatation heart, mostly lt. side; stomach full of blood. Just caudal to 2nd stenotic area aorta an aneurysm size of a cherry contained a thrombus; lower margin shows rupture | Rupture thoracic aortic aneurysm into esophagus & hemorrhage | History rheumatic fever; vague pains legs & shoulders; many attacks fever; disproportion between radial & femoral pulse; murmur between scapulae |
| Brown, J. W.[37] 1934 | Female 30 yrs. | | Atresia aorta beyond origin lt. subclavian & just distal oblit. D. A. | Increased | Bicuspid valve; rt. cusp shows mass yellow friable vegetations arising from root of aorta; projecting into right auricle small unruptured mycotic aneurysm; anomalous vessels originating distal to coarctation | Hypertrophy & dilatation of heart, mostly lt. side. Dilatation ascending aorta; enlarged thyroid gland | Cardiac failure | Dyspnea & swelling legs; B. P. right arm 180/20, left 150/20; systolic B. P. leg 100; upper extremities warm & well colored. Lower extremities cold, bluish & edematous; notching of ribs; violent pulsations neck vessels |
| Hardaway, R. M., and Sawyer, H. P.[38] 1934 | Male 38 yrs. | Stenosis 2 cm. below origin lt. common carotid & lt. subclavian art. 1 cm. above oblit. D. A. | | Increased | No innominate artery; rt. and lt. subclavian arteries originate from arch; anomalous origin of lt. subclavian artery near end descending arch | Hypertrophy & dilatation heart; rib erosion | Heart failure | B. P. 300/140 both arms |
| Rumold, M. J., and Schwartz, E.[39] 1934 | Male 18 yrs. | | Constriction aorta just distal insertion oblit. D. A., atresia | No mention | Bicuspid aortic valves; dissecting aneurysm ascending aorta containing blood clot | Hypertrophy & dilatation aorta; dilatation ascending aorta; patchy myofibrosis | Hemopericardium; rupture walls dissecting aneurysm ascending aorta | Dilated vessels neck, chest & scapular regions |
| Narr, F. C., and Johnson, E. T.[40] 1934 | Male 7 yrs. | | 5 cm. from base of aorta, vessel became narrow & stenosed. At level oblit. D. A. stenosis still present but mod. | No mention | Dissecting aneurysm ascending aorta | About 3 cm. above base of aorta was a rent partially filled with blood clot; at edges of opening 2 large vegetations; ulceration intima ascending aorta | Hemopericardium, rupture ascending aorta | |

TABLE I — *continued*

| Author and year | Age and sex | Infantile type | Adult type | Collateral circulation | Associated anomalies | Secondary findings and other pathology | Cause of death | Clinical data |
|---|---|---|---|---|---|---|---|---|
| Levine, H. D.[41] 1934 | Male 10 mos. | Stenosis aorta between origin lt. subclavian & insertion oblit. D. A. (1.5 cm. cir.) | | No mention | | Hypertrophy & dilatation heart; atelectasis left lung; fibrosis endocardium left ventricle | Cardiac death | |
| Kellogg, F., and Biskind, G. R.[42] 1934 | Male 16 yrs. | | Stenosis 2 cm. below origin lt. subclavian art. & immediately above patent D. A. which admits bristle | Moderate | Anomalous bifid coronary artery behind rt. cusp; bicuspid aortic valve; valve partially destroyed by crumbling vegetations; rupture rt. cusp | Hypertrophy & dilatation lt. ventricle; mycotic aneurysm descending thoracic aorta & superior mesenteric artery | Subacute bacterial endocartitis; terminal bronchopneumonia; recent localized peritonitis | |
| Beatty, J. F.[43] 1934 | Male 22 yrs. | | Atresia aorta level oblit. D. A. | No mention | | Hypertrophy & dilatation heart | | Complete absence pulse in vessels lower limb & abdominal aorta |
| Ballantyne, E. N.[44] 1935 Case 1 | Male 21½ hrs. | Immediately beyond origin lt. subclavian art. aorta narrowed, admits only point of dissecting needle. Stenosis above patent D. A. | | No mention | | Hypertrophy & dilatation heart | Cardiac death | |
| Case 2 | Male 12 wks. | Atresia just beyond origin lt. subclavian art. D. A. patent (3 mm.) | | No mention | Common right ventricle | Hypertrophy rt. & lt. auricles & common ventricle; dilatation ascending aorta | Cardiac death | |
| Case 3 | Male 4 mos. | Stenosis just proximal to oblit. D. A. | | No mention | Foramen ovale anatomically patent; also pars membranacea interventricular septum (8 mm.) | Hypertrophy & dilatation heart | Cardiac death | |
| Farris, H. A.[45] 1935 | Male 11 yrs. | | Stenosis site D. A. (admits probe) D. A. oblit. | Moderate | Bicuspid aortic valve; cusps thickened, contained organized scar tissue about base each valve — ventricular side | Erosion 7th & 8th ribs; hypertrophy & dilatation heart, mostly lt. side; dilatation ascending aorta; obliterative pleuritis — rt.; dilatation & thrombosis descending aorta just beyond atresia | Subarachnoid hemorrhage | X-rays showed absence aortic knob; erosion ribs; pulsating neck vessels |
| Jacobson, C. J.[46] 1935 | Female 35 yrs. | | Stenosis aorta just below insertion oblit. D. A. | Increased | | Hypertrophy & dilatation heart; congestion liver & kidneys | Cardiac death; hemopericardium; perforation diaphragm on concave side arch aorta | |

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| | | | | | | on concave side arch aorta | | |
| Pozzan, A.[47] 1935 | Female 69 yrs. | | Atresia aorta 3 cm. beyond origin lt. subclavian art., aneurysmal niche in superior wall aorta at site oblit. D. A. | Increased | Foramen ovale patent | Hypertrophy & dilatation heart, mostly lt. side; aneurysmal niche in superior wall aorta at site D. A. contains thrombus. Edema legs; cyanosis; gen. arteriosclerosis. 1 cm. below orifices renal arteries aorta transformed into rigid cord oblit. by sclerotic connective tissue & calcium salts. Common iliac, ext. iliacs, hypogastrics on both sides thrombosed, as were the femoral arteries as far as popliteals | Cardiac death | Onset sudden pain in legs at night |

complex anomalies such as a biloculate or triloculate heart, patent foramen ovale, patent interventricular septum, transposition of the arterial trunks, pulmonary atresia and so on.

The case of coarctation we are reporting is unique, not only because of the presence of a double stricture of the aorta, one at the site for infantile coarctation and the second at the location for the adult form, but also on account of coexistent chronic occlusion of the superior vena cava, which necessitated as extensive a collateral venous circulation as was demanded of the arterial tree by the high grade aortic stenosis.

Of all the cases in the available literature since Abbott's summary[1] only 3 in addition to ours exhibit a double stricture at and proximal to the aortic termination of the obliterated ductus. In these cases the descending aorta first narrowed abruptly a slight distance beyond the origin of the left subclavian artery, only to become constricted again at the level of the closed ligamentum arteriosum by a thickened, smooth, internal stenotic ring or complete atresia.[10, 20, 36]

In Table I we have attempted to tabulate all cases of coarctation of the aorta appearing since Abbott's last summary in 1928.

## Report of Case

*Clinical History:* R. C., a 67 year old painter, entered the Multnomah County Hospital in November, 1935, complaining of dysuria, nocturia, frequency, incontinence and constant hematuria. At the age of 22 years he had had gonorrheal urethritis, and a year later a chancre treated by antiluetic therapy which was continued for 4 years. The patient had had lobar pneumonia twice and pleurisy once. When admitted there was no chest pain, palpitation or tachycardia, although he had noted dyspnea on moderate exertion and swelling of the feet and ankles.

*Physical Examination:* The blood pressure was 180/80 left arm, 180/85 right arm; the pulse 98, temperature 98.6° F., and the respirations 20. The patient was of the hyperesthenic type, and lay quietly in bed, although grimacing and grunting every few minutes. The arms, chest and abdomen were covered with varicose veins. The right pupil measured 1.5 mm. in diameter, the left 3.0 mm.; both were round, regular, and did not react to light but showed a fair reaction to accommodation. The neck showed a bilateral, symmetrical, slight lymphadenopathy. The trachea was in the midline. The chest cage was symmetrical with flaring at the costal margins. The expansion was poor but equal. The breath tones were sharp and vesicular. No râles could be heard on ordinary breathing or expiratory cough. The heart presented an aortic configuration. The apex beat was in the sixth interspace 14 cm. from the midline and was localized by a slight thrust. There was slight impairment of resonance to percussion at the upper and lower sternal areas. A blowing systolic murmur was heard over all valve areas. The $M_2$ was weak, while $A_2$ had a loud, ringing metal-

lic sound. The rhythm was regular with an occasional extrasystole. The varicose veins of the arms, chest and abdomen all converged toward the midchest area. The abdomen was rotund, not rigid, and was free from masses or areas of tenderness. The liver was 3 cm. below the costal margin and its edges were smooth. The kidneys and spleen were not palpable. There was tenderness and pain, which radiated down the penis when pressure was made over the bladder area. The prostate was enlarged, irregular, soft and not tender. A firm, nonpainful nodule 0.5 cm. in diameter was palpated posteriorly in the left lobe of the prostate. The reflexes were all very weak except for the biceps, triceps, periosteal and radial, which showed increased reactivity.

*Laboratory Examinations:* Urine: red, alkaline, slightly cloudy with a specific gravity of 1.017, albumin 3 plus, reduction negative, acetone negative, triple phosphates 1 plus, occasional pus cells, red blood cells 1 plus, bacterial 1 plus, and mucus 4 plus.

Blood: hemoglobin 81.8 per cent (Sahli), red blood corpuscles 3,910,000, white blood corpuscles 8500, polymorphonuclears 77 per cent, basophiles 1 per cent, small lymphocytes 5 per cent, mononuclears 4 per cent, staff 12 per cent, degenerated forms 1 per cent, sedimentation rate 45/118. Urea nitrogen 22.5 mg., and alkali reserve 71.8. Kolmer and Kahn tests negative.

*X-Ray:* The first roentgenogram of the chest, made with a portable machine, disclosed nothing beyond slight infiltration apparently along the interlobar fissure on the right side, suggesting the possibility of an interlobar effusion or pneumonic consolidation in the middle or posterior portion of the lower lobe, and definite notching of the inferior margins of the ribs posteriorly. Later chest films showed definite consolidation in the upper lobe of the right lung in addition to notching of the ribs.

*Course of Illness:* A retention catheter was installed and fluids were being forced orally in preparation for cystoscopy when the patient suddenly developed cyanosis of the nose, lips and ears, and a mild edema of the feet. The temperature was 102° F.; respirations 36; pulse 116, full and regular. There was no complaint of pain in the chest. Examination revealed a few, moist, basal râles posteriorly. Under rigorous antipneumonic therapy the temperature slowly fell to 99° F. During the next 3 days the cyanosis disappeared, the respirations decreased to between 12 and 18, and the blood urea nitrogen from 45 to 32 mg. Nevertheless, the patient seemed to be failing and on the 4th day the temperature began to rise, reaching 106° F. on the 6th day of hospitalization. Moist râles were heard all over the chest. Death occurred suddenly on this day.

The clinical diagnoses were: carcinoma of the urinary bladder with retention, cystitis and ascending pyelitis; arteriosclerotic heart disease with hypertrophy and dilatation of all the chambers, regular rhythm with extrasystoles Grade 2-A; neurosyphilis (tabetic type); and bronchopneumonia.

## Postmortem Examination

The body is well nourished and of the muscular type. The neck appears edematous but otherwise is unchanged. The thorax is symmetrical and well developed. Over the chest and abdomen are numerous interlacing, distended superficial veins. The extremities are symmetrical, muscular and well developed.

There is no free fluid in the peritoneal cavity. The urinary bladder is distended with turbid urine. The mucosa is everywhere covered with a dull, gray-green shaggy pseudomembrane. On the posterior wall of the fundus is a papilloma measuring about 2.3 cm. in diameter and capped by a false membrane. The prostate is small and not obstructive. The internal hemorrhoidal plexus of veins shows slight dilatation. The abdominal aorta and the iliac arteries have a smaller than normal caliber.

The lungs are bound down everywhere by fibrous connective tissue adhesions and are freed with difficulty. In the dissection the internal mammary arteries are severed and are found to be abnormally prominent. Their lumens at the point of exit from the mediastinum measure approximately 0.75 cm. in diameter. The intima of both vessels displays considerable arteriosclerosis. The intercostal arteries lie in deep grooves along the inferior borders of the ribs. The grooving is most prominent posteriorly. Over the diaphragm and the base of the pericardium the fibrous adhesions are considerably thickened and contain areas of calcification. The pericardial sac is obliterated by fibrous adhesions and the pericardium is bound externally to all the surrounding structures. The entire lower lobe of the left lung is consolidated. The upper lobe is, for the most part, air-containing; inferiorly it shows focal areas of firmness. The right lung is air-containing, except at the base where a consolidation is noted. At the level of the tracheal bifurcation are three small traction diverticulums, and 5 cm. superiorly are two more. The lower ones are attached to anthracotic and calcified nodes. About the posterior reflections of the diaphragm are seen areas of extreme fibrosis and some calcification. The right adrenal shows at the lateral boundary within the cortex a firm white to yellow area with a few small, white, chalk-like dots in its substance. The right kidney is considerably atrophied, cuts with increased resistance and presents a pitted granular and cystic cortical surface; aberrant arteries go to the lower pole. The left kidney is of moderate size. The cortical surface is smooth. The cut surface shows no distortion of cortical markings. The renal arteries show considerable arteriosclerosis.

The heart is enlarged on both the right and left sides and has a prominent pulmonary conus. It is everywhere of uniform firmness. The maximum transverse diameter is 17 cm. The thickness of the right ventricle at the apex is 0.5 cm., at the base 1.5 cm., and anteri-

orly 1.5 cm. The circumference of the pulmonary ring is 8 cm. The trabeculae carneae of the right ventricle are enlarged and flattened. The anterior papillary muscle is hypertrophied. The right ventricle is considerably dilated. The left ventricle shows pronounced hypertrophy and dilatation. At the apex it measures 1.5 cm. in thickness and at the base 3 cm. The auricular appendages are of normal size and show no gross changes. The pulmonary arteries display well defined arteriosclerosis. The coronary arteries show considerable arteriosclerosis with lumens that are often slit-like but patent. The myocardium of the left ventricle on tangential section exhibits a diffuse patchy replacement fibrosis of the musculature. The endocardium of the left ventricle is slightly thickened and moderately opaque. There is seen a subaortic area of calcification with the formation of an annular concrescence of calcium deposits covered by endothelium. This is found at a distance of 0.5 cm. inferior to the right posterior cusp. Anteriorly it projects upward to involve the anterior cusp and extends into its sinus of Valsalva. The aortic valve is bicuspid. The coronary cusps are congenitally fused, for no nodule of Arantius, raphe or other septum-like structure divides the cusp into two portions. The anterior cusp measures 5 cm. along its free margin. The right posterior cusp measures 4 cm. along the free border. The coronary ostia are of moderate size and lie behind the larger and anteriorly situated cusp. The right coronary orifice lies to the extreme right and anterior half of the larger sinus of Valsalva, while the left is in the extreme posterior angle. The foramen ovale and ductus arteriosus are closed. The aorta is normally situated, bulges slightly to the right and forms a slight aneurysmal dilatation 4.5 cm. in diameter. At the beginning of the transverse arch the aorta becomes constricted and reduced to a diameter of 1 cm. at the site of the infantile isthmus, as compared to 1.8 cm. proximal to the ostium of the subclavian artery. Here the lumen is concentrically constricted by a symmetrical, smooth surfaced ridge or shelf (Figs. 1 and 2). The great size of the left subclavian artery makes it appear to be almost a continuation of the aorta for it too has a diameter of 1.8 cm. Beyond the first coarctation is a slight, but distinct bulbous sacculation of the aorta 2 cm. in diameter. At a point 2 cm. distal to the first coarctation and to the saccule referred to, the descending aortic arch presents externally a shallow annular constriction which coincides internally with an almost complete occlusion of the lumen

by an endothelial covered diaphragm, at the center of which is a minute slit 0.15 cm. in length and barely wide enough to transmit light and water with difficulty. On the medial aspect at the level of the second coarctation the aorta is joined by the ligamentum arteriosum. The descending thoracic aorta just caudal to the second point of stenosis shows a slight diffuse saccular dilatation, the lumen measuring 3.5 cm. in diameter. At the level of the celiac artery the aortic lumen measures 1.8 cm. and at the bifurcation decreases to 1.5 cm. Within an area of 3 square cm., immediately caudal to the lower coarctation, the ostia of ten vessels are seen. These vessels are paired, located symmetrically on the posterior wall and apparently are intercostal arteries. The upper four pair are hypertrophied and dilated, having lumens ranging from 0.3 to 0.7 cm. in diameter. The internal mammary arteries are greatly enlarged and at a point where they leave the mediastinum and course along the chest plate the lumen is found to measure on the right 0.5 cm. and on the left 0.8 cm. The lumen of the left subclavian artery at a distance of 3 cm. from its origin has a diameter of 2 cm. The left common carotid at a similar distance measures 0.8 cm. in diameter. The lumen of the innominate artery is 2.2 cm. in diameter. All arteries leaving the heart and the aortic arch show an advanced degree of arteriosclerosis. No atheromatous degeneration or ulceration is noted. The internal mammary arteries send off branches to the anterior aortic intercostals and communicate freely with the inferior epigastric arteries. The major part of the collateral circulation appears to be between the superior intercostal, arising from the left subclavian artery and the first aortic intercostal (which springs from the aorta just below the second level of the coarctation), the posterior scapularis, the interscapularis, and the subscapularis which, piercing the intercostal spaces from behind, anastomose with the second, third and fourth aortic intercostals.

The superior vena cava is completely occluded from its origin in the right ventricle to its termination in the right jugular and the right subclavian veins (Fig. 3). This obliterative process includes the right innominate vein and one-half of the left innominate vein. The azygos vein is of moderate size and is not dilated. It terminates in the superior vena cava within the area of occlusion. The hemiazygos shows no dilatation. The azygos vein anastomoses freely at its caudal end with the inferior vena cava by way of branches

from the right renal vein and the inferior vena cava. The superficial veins of the thorax and abdomen are considerably dilated and display extensive anastomoses. There is noted a dilatation of the superficial circumflex iliacs, superficial epigastrics, axillary and costo-axillary, mammary venous plexus and thoraco-epigastric veins. There is also considerable dilatation of the deep epigastric, internal mammary, subclavian, intercostal and lateral thoracic veins.

### Microscopic Examination

Microscopic examination of various organs reveals a fibrocaseous tuberculosis of the right suprarenal gland; advanced arteriosclerotic nephropathy; lobar pneumonia in the stage of gray hepatization affecting the lower lobe of the left lung, with bronchopneumonia of the upper lobe; papilloma of the urinary bladder with early carcinomatous changes; diffuse pseudomembranous cystitis; focal purulent prostatitis; patchy myofibrosis of the heart; arteriosclerosis of the aorta; and chronic passive hyperemia of the liver and spleen. Sections of the cord-like superior vena cava show a large amount of hyalinized fibrous tissue containing small vascular channels, some of which are blocked by freshly formed thrombi.

*Anatomical Pathological Diagnoses:* Double coarctation of the aorta, infantile and adult types, the latter producing pronounced stenosis; moderate dilatation of the ascending aorta with beginning saccular aneurysm; extreme dilatation and tortuosity of both subclavian, internal mammary, first aortic intercostal, posterior scapularis, interscapularis, and subscapularis arteries proximal to coarctation, and of the first four pairs of intercostals, deep epigastrics and circumflex iliac arteries distal to coarctation; fusiform aneurysmal dilatation of thoracic aorta immediately caudal to second coarctation; erosion of intercostal grooves of the upper ribs; probable congenital bicuspid aortic valve with partial calcification and stenosis; subaortic annular calcification ring extending into bicuspid aortic valve; healed obliterative pericarditis; hypertrophy and dilatation of the heart; cor pulmonale; pronounced coronary arteriosclerosis; diffuse patchy myofibrosis of the heart; moderate generalized atherosclerosis; pronounced pulmonary arteriosclerosis; old thrombotic occlusion of the superior vena cava and innominate veins; pronounced dilatation of the superficial and deep veins of the abdomen and thorax, lateral thoracic, intercostal, internal mam-

mary, subclavian, and the epigastric veins; slight dilatation of the internal hemorrhoidal venous plexus; lobar pneumonia, left lower lobe; bronchopneumonia, lower right and upper left lobes; acute bronchitis, left; healed obliterative pleuritis, bilateral; calcified empyema pocket at the posterior bases of the pleural cavities; high grade arteriosclerotic nephropathy, especially of the right kidney; calcified tuberculous tracheobronchial lymph nodes; multiple (five) traction diverticulums of the esophagus; old fibrocaseous tuberculosis of the right adrenal; focal purulent prostatitis; papilloma of urinary bladder with early carcinomatous changes; diffuse pseudomembranous cystitis; chronic passive congestion of the viscera; and beginning postsacral decubitus ulcer.

## Discussion and Conclusions

While conforming to the usual picture of aortic coarctation in respect to age, sex, bicuspid aortic valve, development of collateral circulation and notching of the ribs, the case herein reported differs sharply from nearly all others heretofore studied in that there occurs both an infantile and an adult type of coarctation. In one of Abbott's [20] papers is an illustration bearing the title "Double Coarctation of the Aorta," but neither in this paper nor in her review [1] of 200 cases is there any mention of concurrent infantile and adult coarctation. From this it may be inferred that Abbott failed to find any such cases in addition to the one observed by herself. Among the cases of coarctation reported since Abbott's [1] review in 1928 we have encountered only 3 [10, 20, 36] that may be classed as double coarctation. The coexistence of infantile and adult coarctation lends support to the hypothesis of Craigie [48] that the basis of the anomaly lies in an embryological disturbance of formation and involution of the primitive aortic arch.

Another remarkable finding that makes the case reported here unique in the group of aortic coarctation is the concomitant occlusion of the superior vena cava and its innominate tributaries, necessitating the development of a venous collateral return for the head, neck, upper extremities and thorax fully as extensive and intricate as the aortic coarctation demanded of the arterial circulation in order that the abdomen, lower extremities and most of the thorax might receive arterial blood. Thus, of necessity, over the torso both the arterial and the venous blood flowed caudally and in parallel

vessels. There is nothing in the clinical history to indicate when the vena cava became occluded and the only possible clue as to the cause of the fully organized and canalized thrombosis of this vessel is that in conjunction with the pericarditis or pleuritis there may have been a thrombophlebitis of the vena cava superior and its tributaries.

Contributing to the final cardiac decompensation was not only the coarctation of the aorta but the healed pericarditis and pleuritis binding the contents of the thorax to the chest wall and the diaphragm, thus throwing an additional load on the already burdened heart.

Still another cardiac complication deserves mention. In conjunction with the bicuspid aortic valve was a calcified ring which may be classed as a subaortic stenosis, an uncommon yet clinically important cardiac lesion. We are unable to say whether the calcification is part of a degenerative process or the end result of a healed valvulitis, but are inclined to favor the first possibility.

The case reported here illustrates in a remarkable manner the ability of the circulatory system to compensate for obstructions of even major vessels, and further, the reserve strength of the heart which withstood not only the coarctation but also, prior to the last attack, the toxic effects of infection (pneumonia and empyema) and carried well the increased load occasioned by the adhesive pericarditis and pleuritis for an indefinite period of time.

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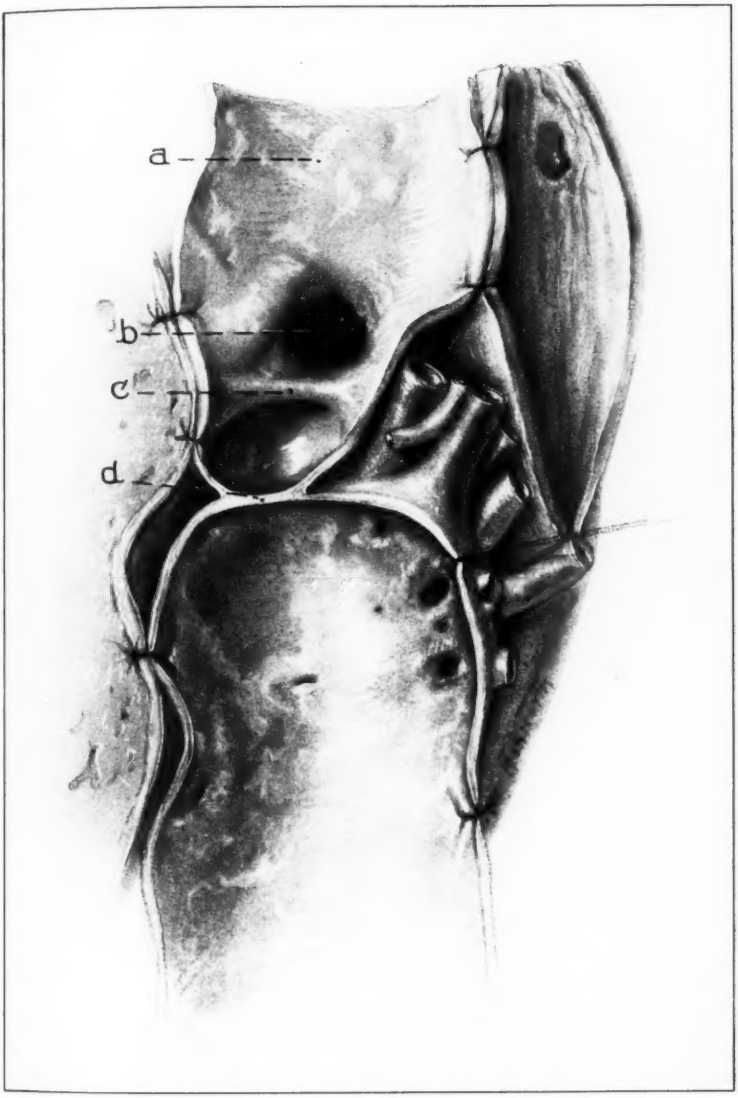
## DESCRIPTION OF PLATES

### PLATE 52

FIG. 1. Actual size drawing of aorta and certain of its branches: 'a' = greatly enlarged left subclavian artery; 'b' = lumen of aortic arch; 'c' = infantile coarctation; 'd' = diaphragm-like adult type coarctation (cut off-center) with only the tiny opening shown on the under surface. Between the two points of stenosis is a sacculated segment of aorta. On the right (pulled back) are the enlarged first four pairs of intercostal arteries.



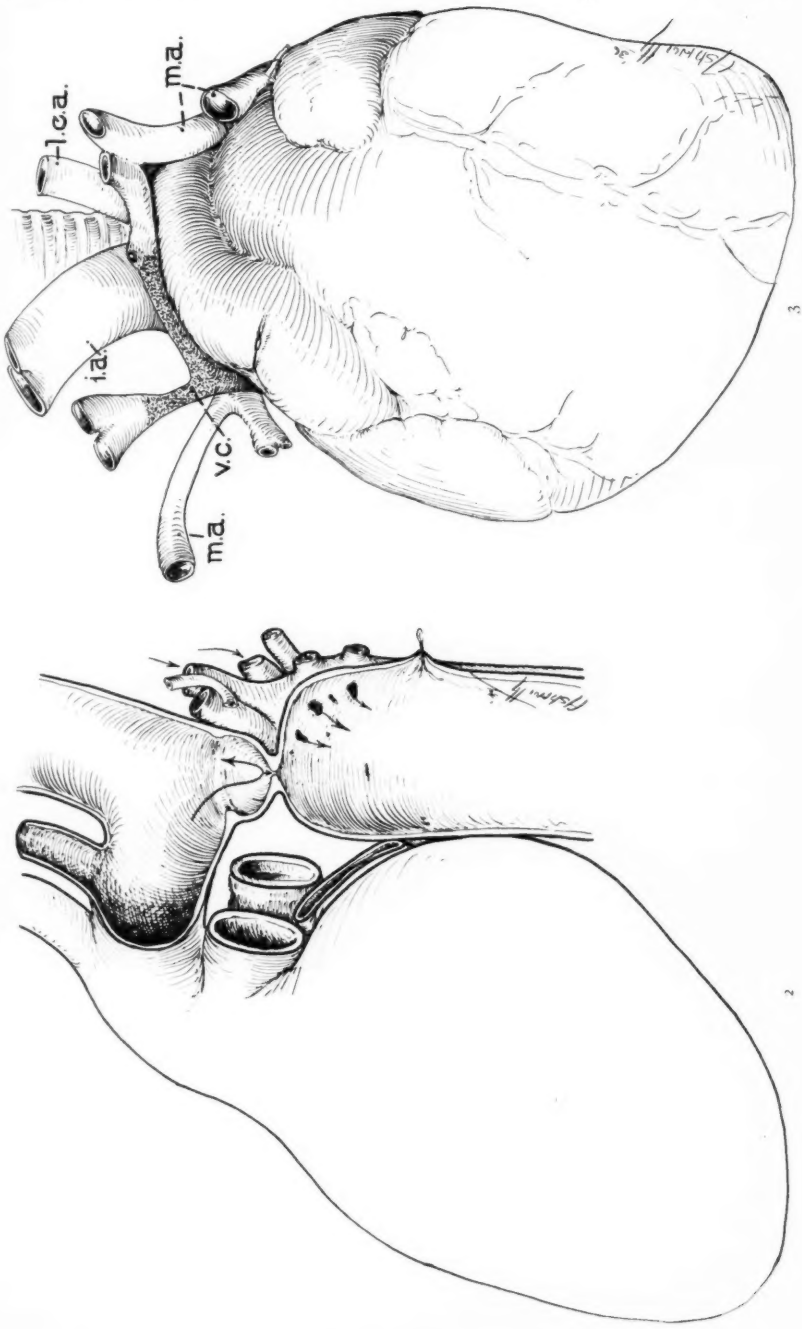

1

Benkwitz and Hunter Infantile and Adult Coarctation of Aorta

PLATE 53

FIG. 2. Diagrammatic drawing illustrating location of coarctations, direction of flow of part of the arterial blood, and comparative size of left common carotid and left subclavian arteries (opened). Compare with Fig. 1.

FIG. 3. Shown diagrammatically are the occluded superior vena cava (v.c.) and its innominate tributaries, innominate (i.a.), left common carotid (l.c.a.) and internal mammary (m.a.) arteries, prominent conus arteriosus and enlarged heart.




Benkwitz and Hunter Infantile and Adult Coarctation of Aorta

# CHRONIC DIFFUSE MESAORTITIS*

## Report of Two Cases of Unusual Type

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It is our purpose to describe the pathology of 2 cases of chronic diffuse mesaortitis in which the aorta presented microscopic changes unlike any hitherto observed in this department or recorded in the available literature.

## Case Reports

Case 1. A. L., P. H. No. 442,294, autopsy No. 11,750, a Jewish male 76 years of age, born in Austria. The past history was incomplete regarding infections, diseases and habits. The illness for which he was admitted to the hospital centered about increasing difficulty in urination over a period of 3 years because of an enlarged prostate. A bilateral, direct inguinal hernia without complications had been present for many years. No symptoms referable to the cardiovascular system were obtained.

*Physical Examination:* The temperature, pulse rate and respirations were normal. The blood pressure was 168/80. There were extensive dental caries and pyorrhea alveolaris. The lungs were clear. The area of cardiac dullness was of normal outline; the sounds were regular and of good quality. Reducible direct inguinal herniae were present. The prostate was considerably enlarged, smooth, firm, mobile and non-tender. There were no other abnormal findings. No evidence of cardiac failure was noted.

*Laboratory Data:* The hemoglobin was 85 per cent, the red blood count 4,600,000. The white cell count was 13,000 with 85 per cent polymorphonuclear leukocytes. The blood Kahn and Wassermann reactions were negative. The blood urea was 20.3 mg. per 100 cc.; the blood sugar 111 gm. per liter. The urine showed a heavy trace of albumin with numerous red cells and an occasional white cell. Electrocardiogram revealed an occasional premature beat arising in the node but there was no indication of heart muscle damage.

X-ray of the chest demonstrated no abnormality of lungs or heart. The aorta appeared tortuous. There was an irregular shadow opposite the third and fourth ribs on the left side, which was interpreted as a diffuse dilatation of the arch of the aorta. X-rays of the urinary tract disclosed no abnormality.

After a vasectomy and a period of 2 weeks with indwelling catheter, a transurethral fulguration of the prostate and neck of the bladder was performed. The temperature rose steadily after this procedure and the patient died 5 days later with signs of consolidation of the lungs.

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*Postmortem Examination*

Autopsy was performed within 1 hour after death. An acute inflammation of the bladder with numerous suppurative foci in the kidneys, thought to be of recent hematogenous origin, were the principal findings. A considerable portion of the prostate remained. It included many nodules of hypertrophic tissue but no evidence of a malignant tumor. No areas of suppuration were present. There was also an extensive confluent lobular pneumonia in both lungs. The heart weighed 310 gm. and presented no abnormalities. The aortic ring measured 7 cm. and the valve appeared delicate but competent.

The aorta was somewhat tortuous as it lay in the body and had lost a considerable part of its elasticity. The approximate circumference after fixation was: ascending aorta 8.1 cm., midportion of the arch 6.6 cm., midthoracic region 5.9 cm., and midabdominal region 3.9 cm. The average normal measurements of the circumference of the aorta in these locations in males between the ages of 71 and 80 years are recorded by Roessle and Roulet[1] as 8.2 cm., 6.7 cm., 5.84 cm., and 4.7 cm. respectively. This aorta, then, could not be considered dilated at any point.

There was a slight diffuse intimal thickening, such as is customarily found at this age, augmented by numerous, irregular, bright yellow zones of poor definition, as well as the usual firm, opaque gray raised plaques which so frequently encircle the orifices of the intercostal and lumbar arteries. Small zones of intimal calcification were sometimes found in the plaques but were nowhere prominent. These changes were more intense in the lower thoracic region than elsewhere. A fine longitudinal wrinkling of the intima was also noted within and bordering the plaques throughout the length of the vessel. No ulceration was present. All of these findings differed in no way from the usual lesions of arteriosclerosis and they were thus so interpreted. They were also found in all of the large arteries throughout the regions of the body examined.

The cross-section of the aorta revealed no changes of note. The media was quite irregular in thickness but not more so than one would expect with the sclerotic lesions. It could not be split apart at any point and no vascular invasion was apparent. The adventitia was not thickened. The gross appearance of the aorta, then, was not unusual in an individual of this age.

### *Microscopic Examination*

After random sections showed a curious alteration of the media, microscopic study was made of the entire length of the aorta. This was done by cutting two long strips, including half the length of the vessel in each and coiling them until they could be accommodated on the ordinary slide. They traversed many intimal plaques formed by hyalinized connective tissue or collections of fat-laden mononuclear cells typical of arteriosclerosis.

The media presented a variegated appearance. At intervals the fibrillar structure was in good part obscured by a diffuse and pronounced cellular infiltration. The great majority of the cells were small lymphocytes, but plasma cells (studied with the orcein-Giemsa and methyl green-pyronin stains) and an occasional polymorphonuclear leukocyte were present as well. An unusual finding was the presence of several multinucleated giant cells of the foreign body type sometimes surrounding refractive fragments which in some instances stained as elastic fibers. None of these cells showed a predilection for the perivascular zones nor were they any more numerous in the outer or the inner portion of the media.

In the areas so involved the fate of the fibers could not be determined with the hematoxylin and eosin stain. A combination of the Weigert elastic tissue and Van Gieson connective tissue stains showed a surprisingly mild degree of destruction. The musculoelastic layer was composed of delicate fibrils and was often indistinct. In the inner third of the media the elastic fibers were regularly disposed and no depletion was noted. In the middle and outer thirds, but particularly in the former, there was a moderate thinning out of elastic, muscle and collagen fibers, but only small areas completely devoid of elastic tissue. The defect was not occupied by collagenous tissue, as is so often the case in luetic aortitis, and there was no proliferation of capillaries to invade the infiltrated portions of the wall. No fat was demonstrated in the media by scharlach R staining. There was no edema.

The adventitia was quite normal, failing to show any thickening or obliterative changes in the nutrient vessels. Occasionally small collections of lymphocytes were present, but not often associated with arterioles.

These changes were found at irregular intervals in both thoracic

and abdominal portions of the aorta and could not be correlated with the degree of intimal sclerosis. Where the cellular infiltration was absent or minimal, the continuity of all medial fibers was preserved, but there was sometimes a mucinous degeneration as shown by the mucicarmine stain. Fine granules identified as calcium salts by the von Kossa stain were disseminated throughout the entire medial coat. The amount of mucinous material and calcium salts was no greater than is observed in the majority of aortas in this age group (Paige and Mott[2]).

No organisms of any description could be demonstrated with the Gram, methylene blue, carbol fuchsin or Levaditi stains.

Sections through the carotid, pulmonary, renal and coeliac arteries failed to reveal similar infiltrations but a reaction quite like that in the aorta was found in the common iliac arteries. Since the lesion was not suspected at the time of autopsy, studies of other arteries could not be made. The medium and small arteries of the viscera were normal.

CASE 2. H. O'C., P. H. No. 439,569, autopsy No. 11,935, a white male, 50 years of age, of non-Jewish heritage, born in Canada and regularly employed as a carpenter. Thirty-five years before his entrance into the hospital he had two attacks of malaria, for which he received quinine. No recurrences were recognized. Typhoid fever, pneumonia, scarlet fever, diphtheria, rheumatic fever and venereal infection were denied. The consumption of alcohol was said to have been slight. No drugs were taken and smoking was confined to a pipe conservatively used. No dietary peculiarities were noted.

The present illness was essentially one of cardiac failure with recurring attacks of precordial pain over a period of 1 year. These were sufficiently severe to be prostrating at times, and toward the latter part of his illness pulmonary edema was pronounced. Medication included luminal, codein, cod liver oil, nitroglycerin, aspirin and ferrous carbonate in the usual dosages.

*Physical Examination:* When first admitted the temperature, pulse and respiration rates were normal. The blood pressure never became elevated. An average reading was 120/70. The lungs were clear in the earlier stages of the illness but later showed signs interpreted as edema. The heart became considerably enlarged and a friction rub was heard. The lower border of the liver was felt just below the costal margin.

*Laboratory Data:* The hemoglobin and red cell count remained within normal range. The white cell count varied from 10,000 to 16,000 with a normal partition. The usual urine and stool examinations were negative. The blood Wassermann reaction was negative. X-rays of the spine were obtained because of the complaint of muscle pains and hypertrophic changes consistent with the diagnosis of rheumatoid arthritis were demonstrated. In this connection the patient was found to have antistreptolysin in the blood, active in a dilution of 1:320. The electrocardiogram suggested considerable myocardial damage. There was a left ventricular preponderance.

The course of illness was one of improvement with rest, although there were numerous recurrences of cardiac and respiratory distress. Shortly before death the temperature rose to 103° F.

### *Postmortem Examination*

Autopsy performed 7½ hours after death disclosed advanced arteriosclerosis of the coronary arteries with occlusion of both branches of the left artery by fibrous tissue and yellow plaques. Extensive recent infarction involved the wall of the left ventricle and the large amount of scar tissue present bore witness to a vascular change of long standing. The heart weighed 460 gm. The aortic ring measured 7 cm. Incidental findings included a bilateral lobular pneumonia, arteriosclerotic scars in the kidneys and hyalinization of a few of the pancreatic islands of Langerhans.

Interest again centered about the changes in the aorta which closely simulated those in the previous case. No tortuosity was noted but the elasticity was patently reduced. There was no dilatation. The circumference after fixation was: ascending aorta 7.6 cm., midportion of the arch 6.4 cm., midthoracic region 5 cm., and midabdominal region 4.4 cm. The intimal changes were minimal but bright yellow, slightly raised plaques and gray fibrous areas of thickening were present, predominantly in the abdominal aorta. Calcification was found only at the site of the obliterated ductus arteriosus and there was no ulceration. On close inspection fine linear wrinkling could be discovered in three small areas, two above and one below the level of the diaphragm.

On section the media appeared to be quite regular and normal in width and no gross degeneration was noted. The vasa vasorum were not prominent in the medial coat. The adventitia was not thickened.

### *Microscopic Examination*

The microscopic appearance of the aorta was even more striking than in the preceding case. Similar stains were employed in the study of cells and fibers and sections were made which included the entire length of the vessel as described above.

The intima was either of normal width or thickened by fibrous tissue showing occasional degeneration, such as is seen in the deeper portions of arteriosclerotic plaques. The media was not reduced in width at any point. The cellular infiltration was diffuse and almost

uniform throughout the length of the aorta. Plasma cells were relatively more numerous and multinucleated giant cells less conspicuous than in the previous case. The latter cells often contained refractive bits of material which sometimes took the stain for elastic tissue. On the whole, there was no increase in the vascularity of the media but at times small vessels of capillary caliber penetrated the entire width of this coat.

Although the cellular infiltration was more pronounced than in the previous case, degeneration of elastic fibers was either only moderate or entirely absent. Where present it occurred in any part of the media but more often was found in the middle third. The intact elastic fibers were moderately wavy and regularly disposed. At no point was scar tissue found in the media, even in areas where there was some depletion of elastic fibers. The adventitia was uniform in thickness. There was no additional collagenous tissue and changes in the nutrient vessels could not be found.

A moderate cellular infiltration was found in the iliac and carotid arteries, but the renal and pulmonary arteries were normal. The left coronary artery was occluded by fibrous tissue thickening of the intima and the media showed only the reduction in width customary in such lesions. The visceral arteries were not significantly altered.

## Discussion

The aortic lesion encountered in these 2 cases is a chronic inflammatory infiltration of the media with minimal degeneration. The intima and adventitia were not involved and no changes could be detected that seemed to be dependent on the medial reaction. Although the cellular invasion was extensive and most striking, the gross alterations of the aorta were insufficient to allow recognition of the lesion without microscopic study. The loss of elasticity can scarcely be ascribed to the medial changes since this is a more common finding in elderly persons. Although dilatation of the aorta was suspected in 1 case by X-ray, this observation was not corroborated by actual measurements of the vessel.

There would seem to be no clinical manifestations of this aortic lesion. No facts in the past history were ascertained suggesting a previous acute infection in the aorta. In the 1st case clinical observers were totally unaware of any damage to the cardiovascular system. The 2nd case presented the picture of progressive

cardiac failure, but this could be referred entirely to sclerosis of the coronary arteries and myocardial infarction. The blood pressure was not elevated in either case. Unfortunately readings were taken only on the upper extremities so that no information is at hand concerning the maintenance of the pressure throughout the entire aorta.

In neither case was there clinical evidence that an infection was present in the body, except as terminal complications of pneumonia and suppurative nephritis. The temperature of each patient was normal on admission. No abnormal cells appeared in the blood, although 1 case, with infarction of the heart, persistently showed a slightly elevated white blood cell count. The erythrocyte sedimentation rate was moderately elevated in the case in which myocardial infarction was present. It was not recorded in the other case.

No clue as to the etiological factor has been gained by morphological studies or by review of the literature. Degenerative and inflammatory changes in the aorta caused by a variety of agents have been described. They are all sufficiently unlike those observed in our 2 cases to justify placing the latter in a distinct category.

The lesion is readily distinguished from the ordinary medial necrosis of the aorta described by Erdheim[3] and others. The typical degeneration classified under this group shows an alteration of the interstitial tissue with the production of a basophilic substance, staining like mucin, and in the more advanced cases depletion of fibers, cystic formation or longitudinal splitting through the wall. There need be little scar tissue and the intima and adventitia are not involved, features which liken the Erdheim necrosis to the lesions in these 2 cases. However, the difference in degree and character of the cellular infiltration is a striking one, and in the cases reported here the degeneration is so mild that it is largely obscured by the massive accumulation of inflammatory cells in the media.

The lesion is decidedly reminiscent of a syphilitic aortitis but there are many factors in which the two conditions are quite dissimilar. In these 2 cases no significant changes could be detected on macroscopic examination, even after the nature of the medial infiltration was known. The intima presented none of the diffuse irregular thickening and deep crevices associated with a luetic infection. Furthermore, alterations were found in various parts of the

aorta and were not limited to the thoracic portion of the vessel, as is more often the case in lues. While destruction of elastic fibers in the media did appear at intervals, the areas were not converted into scar tissue and vascularization of the media was not a feature of any prominence. The types of cells invading the wall are in accord with those found in syphilis, but in the latter disease they frequently form collars about the vasa vasorum and are rarely so uniformly disseminated throughout the entire media. Giant cells have accompanied gummatous degeneration of the aorta but no necrosis was present in conjunction with the large cells in these 2 cases. The appearance of the adventitia was not that of a luetic aortitis since at no point was there thickening, obliterative changes in the nutrient vessels, or perivascular accumulation of cells. As corroborative evidence, the Wassermann reaction in each individual was negative.

Rheumatic aortitis has been described in a variety of forms (Klotz,[4] and Pappenheimer and VonGlahn[5]), none of which is simulated by the lesion here discussed. The cellular infiltration of the intima and subjacent positions of the media with palisades of deeply staining basophilic cells with distorted nuclei, as seen in rheumatic aortitis, was entirely lacking. Adventitial sclerosis and accumulation of typical cells were not found. Other rheumatic lesions were lacking in each case and the history suggested in no way that either individual had suffered from this disease.

Tuberculous foci in the media of the aorta have been present in the form of miliary tubercles or, more rarely, as caseous areas, but no lesion so extensive has been ascribed to the tubercle bacillus. The giant cells present in our cases were not those of Langhans but were of foreign body type, and there was not the slightest suggestion of tubercle formation. Repeated staining with carbol fuchsin failed to reveal any acid-fast bacilli.

A variety of mycotic infections can conceivably stimulate such a cellular response but no fungi could be identified in the wall of the aorta or in any of the viscera.

It has occurred to several pathologists studying these cases that they may represent the chronic phase of an infectious process such as that repeatedly described in the literature as acute aortitis. Influenza is sometimes accompanied by arterial thrombosis, thought to be secondary to bacterial invasion of the wall. The lesion described by Marmorstein[6] in a young female dead of influenza was confined to

the aortic intima over an area about 1.5 cm. in diameter and could be in no way related to the lesion under discussion. Kuskow[7] describes only endothelial changes associated with influenzal infections.

The streptococcus has received considerable attention in relation to alterations in the arterial system, both as isolated case studies and in animal experimentation. Clawson[8] obtained an arteritis, involving all coats, by injecting *Streptococcus viridans* from the blood of patients with rheumatic fever into rabbits and monkeys. The changes were likened to the rheumatic lesions in man and were not those of a possible forerunner of the lesions found in these 2 cases. Benson *et al.*,[9] on the other hand, produced thickening and scarring of the intima, and a mild cellular infiltration of the adventitia and media of rabbit aortas with injections of streptococci, but the lesions were not suggestive of those described above.

The streptococcus is of particular interest since in 1 of the cases a high antistreptolysin titer of the blood was present and a clinical diagnosis of rheumatoid arthritis was made. There have been numerous case reports of focal purulent response in the aorta associated with systemic streptococcal infection, usually occurring in a single isolated area and thought to follow the introduction of organisms via the vasa vasorum or by means of septic emboli lodged on the intimal surface. We are unable to find a reported instance of diffuse phlegmonous mesarteritis in an acute streptococcal disease.

Brody and Smith[10] reviewed the pathology of 44 cases of scarlet fever and 15 cases of possible scarlet fever with the finding of aortic changes in 1 case alone. The capillaries of the adventitia of the aorta are described as having a border of mononuclear cells. No medial infiltration was noted.

The effect of diphtheria toxin on the arterial system of the rabbit has been investigated and reviewed by Duff,[11] who found a purely degenerative lesion in the aorta unaccompanied by inflammatory change. There was occasional calcification and he likens the lesion to the Mönckeberg sclerosis in man.

An acute arteritis occurring in typhoid fever has frequently been observed, with thrombosis and subsequent gangrene of extremities. The extensive literature on this subject is presented by Ophüls.[12] The affected artery is almost always a small one and a pronounced infiltration of the aorta is not described. MacCallum,[13] in discussing the rôle of infections in the pathogenesis of arteriosclerosis, describes

the presence of atheromatous deposits in the intima in a large proportion of typhoid cases in youth but does not record inflammatory changes in the media.

The possible later developments in typhoid fever were investigated by Thayer,[14] who concluded that arterial thrombosis was present because of an underlying arteritis, but this statement is not substantiated by histological studies.

Pneumonia caused by the pneumococcus has been accompanied by acute infections in the arteries, either in the form of small pyogenic foci in the aorta, or in the smaller arteries with thrombosis (McGregor,[15] and Ophüls [12]). The gonococcus has also been present in circumscribed acute infections of the aorta (His [16]), but has never been found with extensive involvement of the aorta.

Localized ulceration, gangrene and perforation of the aorta following infection by *B. anthracis* were described in a single case report (Oliver [17]). In our own laboratory *B. pyocyaneus* was responsible for an acute secondary involvement of a calcareous aortic plaque and the media beneath this plaque was moderately infiltrated with polymorphonuclear leukocytes; there was no inflammatory reaction elsewhere in the aorta (Fish, Hand and Keim [18]).

A review of the autopsy findings in a large variety of infectious diseases by Wiesel [19] includes aortic lesions, notably medial necrosis and edema, but no cellular infiltration accompanied the degeneration and the lesions were usually circumscribed.

Lacking evidence that organisms throughout the media of the aorta initiated the process, which we found as a chronic inflammation and mild degeneration, it is inviting to consider the possibility that a purely allergic reaction is responsible for the entire change. Seegal *et al.*[20] described an intense inflammatory reaction in the pericardium, myocardium and intrapericardial portion of the aorta in sensitized rabbits following intrapericardial injection of the homologous antigen. The aorta in most instances showed some edema, hemorrhage and cellular infiltration in the adventitia. There were also subendothelial collections of polymorphonuclear leukocytes which, in 1 case, extended through the inner half of the wall. When the animals were allowed to live longer after the injection, the polymorphonuclear leukocytes were largely replaced by lymphocytes and mononuclear cells. No organisms were found.

The objections to such a theory, when applied to our cases, are

patent. The Arthus phenomenon, as described by many, is an intense local response at the site of the injection and it is difficult to conceive of a possible portal of entry of a specific antigen which would extensively damage the media of the aorta without intimal or adventitial injury. Furthermore, no positive information regarding a mode of sensitization can be gained from a review of the history in either case. Such an interpretation would be based on pure supposition.

There are numerous chemical substances that are known to affect the aorta in animals. Caution must be used in interpretation of results obtained by experimentation, since they may be quite unique and not at all comparable to the effect of the toxic substances in man. Saltykow [21] describes the lesions produced in rabbits with epinephrin by several investigators. They are usually confined to the media and are characterized by necrosis, scarring and calcification. Again, inflammatory changes are minimal. Saltykow also reviews the work of Fischer who injected hydrochloric acid, phosphoric acid, lactic acid, calcium phosphate, potassium bichromate, uranyl nitrite, chloralamide, mercuric chloride, phlorizine, trypsin, pepsin, iodothyrine, mamma siccata and sodium chloride, obtaining medial lesions but less regularly and not of such a notable character as with epinephrin. The calcification of the media is again a prominent feature and no mention is made of cellular response.

Uranium was found to exert a similar effect by Dominguez,[22] when injected alone or with lead, radium or vanadium, but the latter alone were inert as far as arterial lesions are concerned. The effects again consisted of medial calcification and were quite distinct from the type of response in our 2 cases.

The extensive literature on intoxication by heavy metals, alcohols, tobacco, the halogens, coal tar derivatives and radioactive substances was searched for the production of a medial infiltration or degenerative change in the aorta, without success. The lesion is not reported in man or animal associated with the toxic substances investigated.

## Summary

Two cases presenting diffuse changes in the media of the aorta which do not conform to any recognized type of aortitis are described. They consisted briefly in extensive cellular invasion of a

chronic inflammatory nature with little degeneration of the elastic fibers and no scar formation. The intima and adventitia were not involved. Bacteria were not demonstrated. The medial alteration produced no characteristic changes in the aorta that could be recognized on gross examination. There were no demonstrable clinical manifestations. The histories yielded no clue as to the etiological agent. The literature on medial changes in the aorta is reviewed. No comparable lesion was found.

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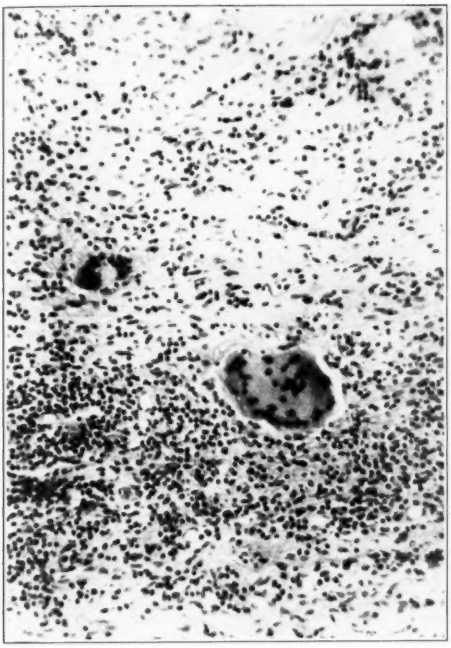
## DESCRIPTION OF PLATES

### PLATE 54

FIG. 1. Case 1. Diffuse infiltration of the media of the aorta, showing two foreign body giant cells. × 170.

FIG. 2. Case 2. Similar diffuse infiltration of the aortic media. × 110.



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2

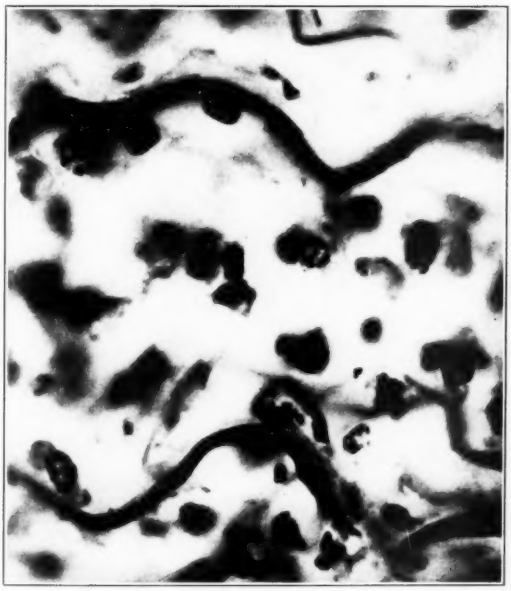
Sproul and Hawthorne Chronic Diffuse Mesaortitis

PLATE 55

FIG. 3. Weigert-Van Gieson stain showing slight disruption of elastic fibers at the site of infiltration of the media. × 250.

FIG. 4. Orcein-Giemsa stain showing plasma cells and lymphocytes between elastic fibers. × 1050.

3



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Sproul and Hawthorne Chronic Diffuse Mesaortitis